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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

BOX PATENT APPLICATION
Assistant Commissioner for Patents
Washington, D.C. 20231

NEEDLE & ROSENBERG, P.C.
Suite 1200, The Candler Building
127 Peachtree Street, N.E.
Atlanta, Georgia 30303-1811

November 21, 2000

Dear Sir:

Transmitted herewith for filing are the specification and claims of the utility patent application of:

Inventor(s): JOHN CHIORINI, ROBERT M. KOTIN, AND BRIAN SAFER

Title of Invention: AAV5 VECTOR AND USES THEREOF

Also enclosed are:

X	20 SHEETS OF	[X] FORMAL DRAWINGS	[] INFORMAL DRAWINGS
	OATH OR DECLARATION OF APPLICANT(S)		
	A POWER OF ATTORNEY		
	A PRELIMINARY AMENDMENT		
	A VERIFIED STATEMENT TO ESTABLISH SMALL ENTITY STATUS UNDER 37 C.F.R. §1.9 AND §1.27		
	A CHECK IN THE AMOUNT OF TO COVER THE FILING FEE.		
	THE COMMISSIONER IS HEREBY AUTHORIZED TO CHARGE ANY ADDITIONAL FEES WHICH MAY BE REQUIRED IN CONNECTION WITH THE FOLLOWING OR CREDIT ANY OVERPAYMENT TO ACCOUNT NO.		
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X	I hereby certify that this correspondence is being placed in the United States Mail as Express Mail No. EL403200449US on the date shown below. Everardo McFarlane — 11/21-2000 DATE		
X	A computer readable form of the sequence listing in compliance with 37 C.F.R. § 1.821(e). The content of the computer readable form of the sequence listing and the sequence listing in the specification of the application as filed are the same.		
	OTHER (IDENTIFY)		

The filing fee is calculated as follows:

<u>CLAIMS AS FILED, LESS ANY CLAIMS CANCELLED BY AMENDMENT</u>

TOTAL CLAIMS = 45 - 20 = 25 x $18.00 =	450.00
INDEPENDENT CLAIMS = 16 - 3 = 13 x $80.00 =	1040.00
BASIC FEE =	$710.00
TOTAL OF ABOVE CALCULATIONS =	$2200.00
REDUCTION BY 1/2 FOR SMALL ENTITY =	
TOTAL FILING FEE =	$2200.00

Respectfully submitted,

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	
Chiorini et al.	
Serial No. Unassigned	Group Art Unit: Unassigned
Filed: Concurrently	Examiner: Unassigned
For: AAV5 VECTOR AND USES THEREOF	

AUTHORIZATION TO TREAT REPLY REQUIRING EXTENSION OF TIME AS INCORPORATING PETITION FOR EXTENSION OF TIME

BOX PATENT APPLICATION
Assistant Commissioner for Patents
Washington, D.C. 20231

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November 21, 2000

Sir:

Pursuant to 37 C.F.R. §1.136(a)(3), the Commissioner is hereby requested and authorized to treat any concurrent or future reply in the above-identified application, requiring a petition for an extension of time for its timely submission, as incorporating a petition for extension of time for the appropriate length of time.

The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. 14-0629.

jc841 U.S. PTO
09/717789
11/21/00

Respectfully submitted,

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PATENT

TO ALL WHOM IT MAY CONCERN:

Be it known that we, JOHN CHIORINI, ROBERT M. KOTIN and BRIAN SAFER, citizens of the United States of America, residing, respectively, at 2604 Loma Street, Silver Spring, MD 20902, 707 Gormley, Rockville, MD 20850 and 1610 Tifton Dr., Silver Springs, MD 20902, U.S.A., have invented new and useful improvements in

AAV5 VECTOR AND USES THEREOF

for which the following is a specification.

AAV5 VECTOR AND USES THEREOF

This application is a continuation of international application PCT/US99/11958 filed on May 28, 1999, which claims priority to U.S. provisional application Serial No. 60/087029 filed on May 28, 1998. The PCT/US99/11958 international application and the 60/087029 provisional patent application are herein incorporated by this reference in their entirety.

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention provides adeno-associated virus 5 (AAV5) and vectors derived therefrom. Thus, the present invention relates to AAV5 vectors for and methods of delivering nucleic acids to cells of subjects.

Background Art

Adeno associated virus (AAV) is a small nonpathogenic virus of the parvoviridae family (for review see 28). AAV is distinct from the other members of this family by its dependence upon a helper virus for replication. In the absence of a helper virus, AAV has been shown to integrate in a locus specific manner into the q arm of chromosome 19 (21). The approximately 5 kb genome of AAV consists of one segment of single stranded DNA of either plus or minus polarity. Physically, the parvovirus virion is non-enveloped and its icosohedral capsid is approximately 20-25 nm in diameter.

To date 8 serologically distinct AAVs have been identified and 6 have been isolated from humans or primates and are referred to as AAV types 1-6 (1). The most extensively studied of these isolates is AAV type 2 (AAV2). The genome of AAV2 is 4680 nucleotides in length and contains two open reading frames (ORFs), the right ORF and the left ORF. The left ORF encodes the non-structural Rep proteins, Rep40,

Rep52, Rep68 and Rep78, which are involved in regulation of replication and transcription in addition to the production of single-stranded progeny genomes (5-8, 11, 12, 15, 17, 19, 21-23, 25, 34, 37-40). Furthermore, two of the Rep proteins have been associated with the preferential integration of AAV genomes into a region of the q arm of human chromosome 19. Rep68/78 have also been shown to possess NTP binding activity as well as DNA and RNA helicase activities. The Rep proteins possess a nuclear localization signal as well as several potential phosphorylation sites. Mutation of one of these kinase sites resulted in a loss of replication activity.

The ends of the genome are short inverted terminal repeats which have the potential to fold into T-shaped hairpin structures that serve as the origin of viral DNA replication. Within the ITR region two elements have been described which are central to the function of the ITR, a GAGC repeat motif and the terminal resolution site (TRS). The repeat motif has been shown to bind Rep when the ITR is in either a linear or hairpin conformation (7, 8, 26).

This binding serves to position Rep68/78 for cleavage at the TRS which occurs in a site- and strand-specific manner. In addition to their role in replication, these two elements appear to be central to viral integration. Contained within the chromosome 19 integration locus is a Rep binding site with an adjacent TRS. These elements have been shown to be functional and necessary for locus specific integration.

The AAV2 virion is a non-enveloped, icosohedral particle approximately 20-25 nm in diameter. The capsid is composed of three related proteins referred to as VP1,2 and 3 which are encoded by the right ORF. These proteins are found in a ratio of 1:1:10 respectively. The capsid proteins differ from each other by the use of alternative splicing and an unusual start codon. Deletion analysis of has shown that removal or alteration of AAV2 VP1 which is translated from an alternatively spliced message results in a reduced yield of infections particles (15, 16, 38). Mutations within the VP3

coding region result in the failure to produce any single-stranded progeny DNA or infectious particles (15, 16, 38).

The following features of the characterized AAVs have made them attractive vectors for gene transfer (16). AAV vectors have been shown *in vitro* to stably integrate into the cellular genome; possess a broad host range; transduce both dividing and non dividing cells *in vitro* and *in vivo* (13, 20, 30, 32) and maintain high levels of expression of the transduced genes (41). Viral particles are heat stable, resistant to solvents, detergents, changes in pH, temperature, and can be concentrated on CsCl gradients (1,2). Integration of AAV provirus is not associated with any long term negative effects on cell growth or differentiation (3,42). The ITRs have been shown to be the only cis elements required for replication, packaging and integration (35) and may contain some promoter activities (14).

AAV2 was originally thought to infect primate and non-primate cell types provided the appropriate helper virus was present. However, the inability of AAV2 to infect certain cell types is now known to be due to the particular cellular tropism exhibited by the AAV2 virus. Recent work has shown that some cell lines are transduced very poorly by AAV2 (30). Binding studies have indicated that heparin sulfate proteoglycans are necessary for high efficiency transduction with AAV2. AAV5 is a unique member of the parvovirus family. The present DNA hybridization data indicate a low level of homology with the published AAV1-4 sequences (31). The present invention shows that, unlike AAV2, AAV5 transduction is not effected by heparin as AAV2 is and therefore will not be restricted to the same cell types as AAV2.

The present invention provides a vector comprising the AAV5 virus or a vector comprising subparts of the virus, as well as AAV5 viral particles. While AAV5 is similar to AAV2, the two viruses are found herein to be physically and genetically distinct. These differences endow AAV5 with some unique properties and advantages which better suit it as a vector for gene therapy. For example, one of the limiting

features of using AAV2 as a vector for gene therapy is production of large amounts of virus. Using standard production techniques, AAV5 is produced at a 10-50 fold higher level compared to AAV2. Because of its unique TRS site and rep proteins, AAV5 should also have a distinct integration locus compared to AAV2.

Furthermore, as shown herein, AAV5 capsid protein, again surprisingly, is distinct from AAV2 capsid protein and exhibits different tissue tropism, thus making AAV5 capsid-containing particles suitable for transducing cell types for which AAV2 is unsuited or less well-suited. AAV2 and AAV5 have been shown to be serologically distinct and thus, in a gene therapy application, AAV5, and AAV5-derived vectors, would allow for transduction of a patient who already possess neutralizing antibodies to AAV2 either as a result of natural immunological defense or from prior exposure to AAV2 vectors. Another advantage of AAV5 is that AAV5 cannot be rescued by other serotypes. Only AAV5 can rescue the integrated AAV5 genome and effect replication, thus avoiding unintended replication of AAV5 caused by other AAV serotypes. Thus, the present invention, by providing these new recombinant vectors and particles based on AAV5 provides a new and highly useful series of vectors.

SUMMARY OF THE INVENTION

The present invention provides a nucleic acid vector comprising a pair of adeno-associated virus 5 (AAV5) inverted terminal repeats and a promoter between the inverted terminal repeats.

The present invention further provides an AAV5 particle containing a vector comprising a pair of AAV2 inverted terminal repeats.

Additionally, the instant invention provides an isolated nucleic acid comprising the nucleotide sequence set forth in SEQ ID NO:1 (AAV5 genome). Furthermore, the

present invention provides an isolated nucleic acid consisting essentially of the nucleotide sequence set forth in SEQ ID NO:1 (AAV5 genome).

The present invention provides an isolated nucleic acid encoding an AAV5 Rep protein, for example, the nucleic acid as set forth in SEQ ID NO:10. Additionally provided is an isolated full-length AAV5 Rep protein or a unique fragment thereof. Additionally provided is an isolated AAV5 Rep 40 protein having the amino acid sequence set forth in SEQ ID NO:12, or a unique fragment thereof. Additionally provided is an isolated AAV5 Rep 52 protein having the amino acid sequence set forth in SEQ ID NO:2, or a unique fragment thereof. Additionally provided is an isolated AAV5 Rep 68 protein, having the amino acid sequence set forth in SEQ ID NO:14 or a unique fragment thereof. Additionally provided is an isolated AAV5 Rep 78 protein having the amino acid sequence set forth in SEQ ID NO:3, or a unique fragment thereof. The sequences for these proteins are provided below in the Sequence Listing and elsewhere in the application where the proteins are described.

The present invention further provides an isolated AAV5 capsid protein, VP1, having the amino acid sequence set forth in SEQ ID NO:4, or a unique fragment thereof. Additionally provided is an isolated AAV5 capsid protein, VP2, having the amino acid sequence set forth in SEQ ID NO:5, or a unique fragment thereof. Also provided is an isolated AAV5 capsid protein, VP3, having the amino acid sequence set forth in SEQ ID NO:6, or a unique fragment thereof.

The present invention additionally provides an isolated nucleic acid encoding AAV5 capsid protein, for example, the nucleic acid set forth in SEQ ID NO:7, or a unique fragment thereof.

The present invention further provides an AAV5 particle comprising a capsid protein consisting essentially of the amino acid sequence set forth in SEQ ID NO:4, or a unique fragment thereof.

Additionally provided by the present invention is an isolated nucleic acid comprising an AAV5 p5 promoter having the nucleic acid sequence set forth in SEQ ID NO:18, or a unique fragment thereof.

The instant invention provides a method of screening a cell for infectivity by AAV5 comprising contacting the cell with AAV5 and detecting the presence of AAV5 in the cells.

The present invention further provides a method of delivering a nucleic acid to a cell comprising administering to the cell an AAV5 particle containing a vector comprising the nucleic acid inserted between a pair of AAV inverted terminal repeats, thereby delivering the nucleic acid to the cell.

The present invention also provides a method of delivering a nucleic acid to a subject comprising administering to a cell from the subject an AAV5 particle comprising the nucleic acid inserted between a pair of AAV inverted terminal repeats, and returning the cell to the subject, thereby delivering the nucleic acid to the subject.

The present invention also provides a method of delivering a nucleic acid to a cell in a subject comprising administering to the subject an AAV5 particle comprising the nucleic acid inserted between a pair of AAV inverted terminal repeats, thereby delivering the nucleic acid to a cell in the subject.

The instant invention further provides a method of delivering a nucleic acid to a cell in a subject having antibodies to AAV2 comprising administering to the subject an AAV5 particle comprising the nucleic acid, thereby delivering the nucleic acid to a cell in the subject.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows Heparin inhibition results. Cos cells were plated in 12 well dishes at $5X10^4$ cells per well. Serial dilutions of AAV2 or AAV5 produced and purified as previously described and supplemented with $5X10^5$ particles of wt adenovirus were incubated for 1 hr at Rt in the presence of 20 μg/ml heparin (sigma). Following this incubation the virus was added to the cells in 400 μl of media for 1 hr after which the media was removed, the cells rinsed and fresh media added. After 24 hrs the plates were stained for Bgal activity.

Figure 2 shows AAV2 and AAV5 vector and helper complementation. Recombinant AAV particles were produced as previously described using a variety of vector and helper plasmids as indicated the bottom of the graph. The vector plasmids contained the Bgal gene with and RSV promoter and flanked by either AAV2 ITRs (2ITR) or AAV5 ITRs (5ITR). The helper plasmids tested contained either AAV2 Rep and cap genes (2repcap) AAV5 rep and cap genes with or without an SV40 promoter (5repcapA and 5repcapb respectively) only the AAV2 rep gene (2rep) in varying amounts (1) or (.5) or an empty vector (pUC). The resulting AAV particles were then titered on cos cells. AAV particles were only produced when the same serotype of ITR and Rep were present.

Figure 3 shows AAV2 and AAV5 tissue tropism. Transduction of a variety of cell types indicated that AAV2 and AAV5 transduce cells with different efficiencies. Equal number of either AAV2 or AAV5 particles were used to transduce a variety of cell types and the number of bgal positive cells is reported.

Figure 4 is a sequence comparison of the AAV2 genome and the AAV5 genome.

Figure 5 is a sequence comparison of the AAV2 VP1 capsid protein and the AAV5 VP1 capsid protein.

Figure 6 is a sequence comparison of the AAV2 rep 78 protein and the AAV5 rep 78 protein.

Figure 7 shows the transduction of airway epithelial cells by AAV5. Primary airway epithelial cells were cultured and plated. Cells were transducted with an equivalent number of rAAV2 or rAAV5 particles containing a nuclear localized β-gal transgene with 50 particles of virus/cell (MOI 50) and continued in culture for 10 days. β-gal activity was determined and the relative transduction efficiency compared. AAV5 transduced these cells 50- fold more efficiently than AAV2. This is the first time apical cells or cells exposed to the air have been shown to be infected by a gene therapy agent.

Figure 8 shows transduction of striated muscle by AAV5. Chicken myoblasts were cultured and plated. Cells were allowed to fuse and then transduced with a similar number of particles of rAAV2 or rAAV5 containing a nuclear localized β-gal transgene after 5 days in culture. The cells were stained for β-gal activity and the relative transduction efficiency compared. AAV5 transduced these cells approximately 16 fold more efficiently than AAV2.

Figure 9 shows transduction of rat brain explants by AAV5. Primary neonatal rat brain explants were prepared. After 7 days in culture, cells were transduced with a similar number of particles of rAAV5 containing a nuclear localized β-gal transgene. After 5 days in culture, the cells were stained for β-gal activity. Transduction was detected in a variety of cell types including astrocytes, neuronal cells and glial cells.

Figure 10 shows transduction of human umbilical vein endothelial cells by AAV5. Human umbilical vein endothelial cells were cultured and plated. Cells were

transduced with rAAV2 or rAAV5 containing a nuclear localized β-gal transgene with 10 particles of virus/ cell (MOI 5) in minimal media then returned to complete media. After 24 hrs in culture, the cells were stained for β-gal activity and the relative transduction efficiency compared. As shown in AAV5 transduced these cell 5-10 fold more efficiently than AAV2.

DETAILED DESCRIPTION OF THE INVENTION

As used in the specification and in the claims, "a" can mean one or more, depending upon the context in which it is used. The terms “having” and “comprising” are used interchangeably herein, and signify open ended meaning.

The present application provides a recombinant adeno-associated virus 5 (AAV5). This virus has one or more of the characteristics described below. The compositions of the present invention do not include wild-type AAV5. The methods of the present invention can use either wild-type AAV5 or recombinant AAV5-based delivery.

The present invention provides novel AAV5 particles, recombinant AAV5 vectors, recombinant AAV5 virions and novel AAV5 nucleic acids and polypeptides. An AAV5 particle is a viral particle comprising an AAV5 capsid protein. A recombinant AAV5 vector is a nucleic acid construct that comprises at least one unique nucleic acid of AAV5. A recombinant AAV5 virion is a particle containing a recombinant AAV5 vector, wherin the particle can be either an AAV5 particle as described herein or a non-AAV5 particle. Alternatively, the recombinant AAV5 virion is an AAV5 particle containing a recombinant vector, wherein the vector can be either an AAV5 vector as described herein or a non-AAV5 vector. These vectors, particles, virions, nucleic acids and polypeptides are described below.

The present invention provides the nucleotide sequence of the AAV5 genome and vectors and particles derived therefrom. Specifically, the present invention provides a nucleic acid vector comprising a pair of AAV5 inverted terminal repeats (ITRs) and a promoter between the inverted terminal repeats. While the rep proteins of AAV2 and AAV5 will bind to either a type 2 ITR or a type 5 ITR, efficient genome replication only occurs when type 2 Rep replicates a type 2 ITR and a type 5 Rep replicates a type 5 ITR. This specificity is the result of a difference in DNA cleavage specificity of the two Reps which is necessary for replication. AAV5 Rep cleaves at CGGT^GTGA (SEQ ID NO: 21) and AAV2 Rep cleaves at CGGT^TGAG (SEQ ID NO: 22) (Chiorini et al., 1999. J. Virol. 73 (5) 4293-4298). Mapping of the AAV5 ITR terminal resolution site (TRS) identified this distinct cleavage site, CGGT^GTGA, which is absent from the ITRs of other AAV serotypes. Therefore, the minimum sequence necessary to distinguish AAV5 from AAV2 is the TRS site where Rep cleaves in order to replicate the virus. Examples of the type 5 ITRs are shown in SEQ ID NO: 19 and SEQ ID NO: 20, AAV5 ITR “flip” and AAV5 “flop”, respectively. Minor modifications in an ITR of either orientation are contemplated and are those that will not interfere with the hairpin structure formed by the AAV5 ITR as described herein and known in the art. Furthermore, to be considered within the term "AAV5 ITR" the nucleotide sequence must retain one or more features described herein that distinguish the AAV5 ITR from the ITRs of other serotypes, e.g. it must retain the Rep binding site described herein.

The D- region of the AAV5 ITR (SEQ ID NO: 23), a single stranded region of the ITR, inboard of the TRS site, has been shown to bind a factor which depending on its phosphorylation state correlates with the conversion of the AAV from a single stranded genome to a transcriptionally active form that allows for expression of the viral DNA. This region is conserved between AAV2, 3, 4,and 6 but is divergent in AAV5. The D+ region is the reverse complement of the D- region.

The promoter can be any desired promoter, selected by known considerations, such as the level of expression of a nucleic acid functionally linked to the promoter and the cell type in which the vector is to be used. That is, the promoter can be tissue/cell-specific. Promoters can be prokaryotic, eukaryotic, fungal, nuclear, mitochondrial, viral or plant promoters. Promoters can be exogenous or endogenous to the cell type being transduced by the vector. Promoters can include, for example, bacterial promoters, known strong promoters such as SV40 or the inducible metallothionein promoter, or an AAV promoter, such as an AAV p5 promoter. Additionally, chimeric regulatory promoters for targeted gene expression can be utilized. Examples of these regulatory systems, which are known in the art, include the tetracycline based regulatory system which utilizes the tet transactivator protein (tTA), a chimeric protein containing the VP16 activation domain fused to the tet repressor of *Escherichia coli,* the IPTG based regulatory system, the CID based regulatory system, and the Ecdysone based regulatory system (44). Other promoters include promoters derived from actin genes, immunoglobulin genes, cytomegalovirus (CMV), adenovirus, bovine papilloma virus, adenoviral promoters, such as the adenoviral major late promoter, an inducible heat shock promoter, respiratory syncytial virus, Rous sarcomas virus (RSV), etc., specifically, the promoter can be AAV2 p5 promoter or AAV5 p5 promoter. More specifically, the AAV5 p5 promoter can be about same location in SEQ ID NO: 1 as the AAV2 p5 promoter, in the corresponding AAV2 published sequence. Additionally, the p5 promoter may be enhanced by nucleotides 1-130 of SEQ ID NO:1. Furthermore, smaller fragments of p5 promoter that retain promoter activity can readily be determined by standard procedures including, for example, constructing a series of deletions in the p5 promoter, linking the deletion to a reporter gene, and determining whether the reporter gene is expressed, *i.e.*, transcribed and/or translated. The promoter can be the promoter of any of the AAV serotypes, and can be the p19 promoter (SEQ ID NO: 16) or the p40 promoter set forth in the sequence listing as SEQ ID NO: 17.

It should be recognized that any errors in any of the nucleotide sequences disclosed herein can be corrected, for example, by using the hybridization procedure

described below with various probes derived from the described sequences such that the coding sequence can be reisolated and resequenced. Rapid screening for point mutations can also be achieved with the use of polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) (43). The corresponding amino acid sequence can then be corrected accordingly.

The AAV5-derived vector of the invention can further comprise a heterologous nucleic acid functionally linked to the promoter. By "heterologous nucleic acid" is meant that any heterologous or exogenous nucleic acid, i.e. not normally found in wild-type AAV5 can be inserted into the vector for transfer into a cell, tissue or organism. By "functionally linked" is meant that the promoter can promote expression of the heterologous nucleic acid, as is known in the art, and can include the appropriate orientation of the promoter relative to the heterologous nucleic acid. Furthermore, the heterologous nucleic acid preferably has all appropriate sequences for expression of the nucleic acid. The nucleic acid can include, for example, expression control sequences, such as an enhancer, and necessary information processing sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences.

The heterologous nucleic acid can encode beneficial proteins or polypeptides that replace missing or defective proteins required by the cell or subject into which the vector is transferred or can encode a cytotoxic polypeptide that can be directed, *e.g.*, to cancer cells or other cells whose death would be beneficial to the subject. The heterologous nucleic acid can also encode antisense RNAs that can bind to, and thereby inactivate, mRNAs made by the subject that encode harmful proteins. The heterologous nucleic acid can also encode ribozymes that can effect the sequence-specific inhibition of gene expression by the cleavage of mRNAs. In one embodiment, antisense polynucleotides can be produced from a heterologous expression cassette in an AAV5 vector construct where the expression cassette contains a sequence that promotes cell-type specific expression (Wirak *et al.*, *EMBO* 10:289

(1991)). For general methods relating to antisense polynucleotides, see *Antisense RNA and DNA*, D. A. Melton, Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1988).

Examples of heterologous nucleic acids which can be administered to a cell or subject as part of the present AAV5 vector can include, but are not limited to the following: nucleic acids encoding secretory and nonsecretory proteins, nucleic acids encoding therapeutic agents, such as tumor necrosis factors (TNF), such as TNF-α; interferons, such as interferon-α, interferon-β, and interferon-γ; interleukins, such as IL-1, IL-1β, and ILs -2 through -14; GM-CSF; adenosine deaminase; cellular growth factors, such as lymphokines; soluble CD4; Factor VIII; Factor IX; T-cell receptors; LDL receptor; ApoE; ApoC; alpha-1 antitrypsin; ornithine transcarbamylase (OTC); cystic fibrosis transmembrane receptor (CFTR); insulin; Fc receptors for antigen binding domains of antibodies, such as immunoglobulins; anit-HIV decoy tar elements; and antisense sequences which inhibit viral replication, such as antisense sequences which inhibit replication of hepatitis B or hepatitis non-A, non-B virus. The nucleic acid is chosen considering several factors, including the cell to be transfected. Where the target cell is a blood cell, for example, particularly useful nucleic acids to use are those which allow the blood cells to exert a therapeutic effect, such as a gene encoding a clotting factor for use in treatment of hemophilia. Another target cell is the lung airway cell, which can be used to administer nucleic acids, such as those coding for the cystic fibrosis transmembrane receptor, which could provide a gene therapeutic treatment for cystic fibrosis. Other target cells include muscle cells where useful nucleic acids, such as those encoding cytokines and growth factors, can be transduced and the protein the nucleic acid encodes can be expressed and secreted to exert its effects on other cells, tissues and organs, such as the liver. Furthermore, the nucleic acid can encode more than one gene product, limited only, if the nucleic acid is to be packaged in a capsid, by the size of nucleic acid that can be packaged.

Furthermore, suitable nucleic acids can include those that, when transferred into a primary cell, such as a blood cell, cause the transferred cell to target a site in the body where that cell's presence would be beneficial. For example, blood cells such as TIL cells can be modified, such as by transfer into the cell of a Fab portion of a monoclonal antibody, to recognize a selected antigen. Another example would be to introduce a nucleic acid that would target a therapeutic blood cell to tumor cells. Nucleic acids useful in treating cancer cells include those encoding chemotactic factors which cause an inflammatory response at a specific site, thereby having a therapeutic effect.

Cells, particularly blood cells, muscle cells, airway epithelial cells, brain cells and endothelial cells having such nucleic acids transferred into them can be useful in a variety of diseases, syndromes and conditions. For example, suitable nucleic acids include nucleic acids encoding soluble CD4, used in the treatment of AIDS and α-antitrypsin, used in the treatment of emphysema caused by α-antitrypsin deficiency. Other diseases, syndromes and conditions in which such cells can be useful include, for example, adenosine deaminase deficiency, sickle cell deficiency, brain disorders such as Alzheimer's disease, thalassemia, hemophilia, diabetes, phenylketonuria, growth disorders and heart diseases, such as those caused by alterations in cholesterol metabolism, and defects of the immune system.

As another example, hepatocytes can be transfected with the present vectors having useful nucleic acids to treat liver disease. For example, a nucleic acid encoding OTC can be used to transfect hepatocytes (*ex vivo* and returned to the liver or *in vivo*) to treat congenital hyperammonemia, caused by an inherited deficiency in OTC. Another example is to use a nucleic acid encoding LDL to target hepatocytes *ex vivo* or *in vivo* to treat inherited LDL receptor deficiency. Such transfected hepatocytes can also be used to treat acquired infectious diseases, such as diseases resulting from a viral infection. For example, transduced hepatocyte precursors can be used to treat viral hepatitis, such as hepatitis B and non-A, non-B hepatitis, for example by transducing the hepatocyte precursor with a nucleic acid encoding an antisense RNA that inhibits

viral replication. Another example includes transferring a vector of the present invention having a nucleic acid encoding a protein, such as α-interferon, which can confer resistance to the hepatitis virus.

For a procedure using transfected hepatocytes or hepatocyte precursors, hepatocyte precursors having a vector of the present invention transferred in can be grown in tissue culture, removed from the tissue culture vessel, and introduced to the body, such as by a surgical method. In this example, the tissue would be placed directly into the liver, or into the body cavity in proximity to the liver, as in a transplant or graft. Alternatively, the cells can simply be directly injected into the liver, into the portal circulatory system, or into the spleen, from which the cells can be transported to the liver via the circulatory system. Furthermore, the cells can be attached to a support, such as microcarrier beads, which can then be introduced, such as by injection, into the peritoneal cavity. Once the cells are in the liver, by whatever means, the cells can then express the nucleic acid and/or differentiate into mature hepatocytes which can express the nucleic acid.

The AAV5-derived vector can include any normally occurring AAV5 sequences in addition to an ITR and promoter. Examples of vector constructs are provided below.

The present vector or AAV5 particle or recombinant AAV5 virion can utilize any unique fragment of these present AAV5 nucleic acids, including the AAV5 nucleic acids set forth in SEQ ID NOS: 1 and 7-11, 13, 15, 16, 17, and 18. To be unique, the fragment must be of sufficient size to distinguish it from other known sequences, most readily determined by comparing any nucleic acid fragment to the nucleotide sequences of nucleic acids in computer databases, such as GenBank. Such comparative searches are standard in the art. Typically, a unique fragment useful as a primer or probe will be at least about 8 or 10, preferable at least 20 or 25 nucleotides in length, depending upon the specific nucleotide content of the sequence. Additionally, fragments can be, for example, at least about 30, 40, 50, 75, 100, 200 or 500 nucleotides in length and can

encode polypeptides or be probes. The nucleic acid can be single or double stranded, depending upon the purpose for which it is intended. Where desired, the nucleic acid can be RNA.

The present invention further provides an AAV5 capsid protein to contain the vector. In particular, the present invention provides not only a polypeptide comprising all three AAV5 coat proteins, *i.e.*, VP1, VP2 and VP3, but also a polypeptide comprising each AAV5 coat protein individually, SEQ ID NOS: 4, 5, and 6, respectively. Thus an AAV5 particle comprising an AAV5 capsid protein comprises at least one AAV5 coat protein VP1, VP2 or VP3. An AAV5 particle comprising an AAV5 capsid protein can be utilized to deliver a nucleic acid vector to a cell, tissue or subject. For example, the herein described AAV5 vectors can be encapsidated in an AAV5 capsid-derived particle and utilized in a gene delivery method. Furthermore, other viral nucleic acids can be encapsidated in the AAV5 particle and utilized in such delivery methods. For example, an AAV1, 2,3,4,or 6 vector (e.g. AAV1,2,3,4,or 6 ITR and nucleic acid of interest)can be encapsidated in an AAV5 particle and administered. Furthermore, an AAV5 chimeric capsid incorporating both AAV2 capsid and AAV5 capsid sequences can be generated, by standard cloning methods, selecting regions from the known sequences of each protein as desired. For example, particularly antigenic regions of the AAV2 capsid protein can be replaced with the corresponding region of the AAV5 capsid protein. In addition to chimeric capsids incorporating AAV2 capsid sequences, chimeric capsids incorporating AAV1, 3, 4, or 6 and AAV5 capsid sequences can be generated, by standard cloning methods, selecting regions from the known sequences of each protein as desired.

The capsids can also be modified to alter their specific tropism by genetically altering the capsid to encode a specific ligand to a cell surface receptor. Alternatively, the capsid can be chemically modified by conjugating a ligand to a cell surface receptor. By genetically or chemically altering the capsids, the tropism can be modified to direct AAV5 to a particular cell or population of cells. The capsids can

also be altered immunologically by conjugating the capsid to an antibody that recognizes a specific protein on the target cell or population of cells.

The capsids can also be assembled into empty particles by expression in mammalian, bacterial, fungal or insect cells. For example, AAV2 particles are known to be made from VP3 and VP2 capsid proteins in baculovirus. The same basic protocol can produce an empty AAV5 particle comprising an AAV5 capsid protein.

The herein described recombinant AAV5 nucleic acid derived vector can be encapsidated in an AAV particle. In particular, it can be encapsidated in an AAV1 particle, an AAV2 particle, an AAV3 particle, an AAV4 particle, an AAV5 particle or an AAV6 particle, a portion of any of these capsids, or a chimeric capsid particle as described above, by standard methods using the appropriate capsid proteins in the encapsidation process, as long as the nucleic acid vector fits within the size limitation of the particle utilized. The encapsidation process itself is standard in the art. The AAV5 replication machinery, i.e. the rep initiator proteins and other functions required for replication, can be utilized to produce the AAV5 genome that can be packaged in an AAV1, 2, 3, 4, 5 or 6 capsid.

The recombinant AAV5 virion containing a vector can also be produced by recombinant methods utilizing multiple plasmids. In one example, the AAV5 rep nucleic acid would be cloned into one plasmid, the AAV5 ITR nucleic acid would be cloned into another plasmid and the AAV1, 2, 3, 4, 5 or 6 capsid nucleic acid would be cloned on another plasmid. These plasmids would then be introduced into cells. The cells that were efficiently transduced by all three plasmids, would exhibit specific integration as well as the ability to produce AAV5 recombinant virus. Additionally, two plasmids could be used where the AAV5 rep nucleic acid would be cloned into one plasmid and the AAV5 ITR and AAV5 capsid would be cloned into another plasmid. These plasmids would then be introduced into cells. The cells that were efficiently

transduced by both plasmids, would exhibit specific integration as well as the ability to produce AAV5 recombinant virus.

An AAV5 capsid polypeptide encoding the entire VP1, VP2, and VP3 polypeptide can overall has greater than 56% homology to the polypeptide having the amino acid sequence encoded by nucleotides in SEQ ID NOS:7,8 and 9, as shown in figures 4 and 5. The capsid protein can have about 70% homology, about 75% homology, 80% homology, 85% homology, 90% homology, 95% homology, 98% homology, 99% homology, or even 100% homology to the protein having the amino acid sequence encoded by the nucleotides set forth in SEQ ID NOS:7, 8 or 9. The percent homology used to identify proteins herein, can be based on a nucleotide-by-nucleotide comparison or more preferable is based on a computerized algorithm as described herein. Variations in the amino acid sequence of the AAV5 capsid protein are contemplated herein, as long as the resulting particle comprising an AAV5 capsid protein remains antigenically or immunologically distinct from AAV1, AAV2, AAV3, AAV4 or AAV6 capsid, as can be routinely determined by standard methods. Specifically, for example, ELISA and Western blots can be used to determine whether a viral particle is antigenically or immunologically distinct from AAV2 or the other serotypes. Furthermore, the AAV5 particle preferably retains tissue tropism distinction from AAV2, such as that exemplified in the examples herein. An AAV5 chimeric particle comprising at least one AAV5 coat protein may have a different tissue tropism from that of an AAV5 particle consisting only of AAV5 coat proteins, but is still distinct from the tropism of an AAV2 particle.

The invention further provides a recombinant AAV5 virion, comprising an AAV5 particle containing, *i.e.*, encapsidating, a vector comprising a pair of AAV5 inverted terminal repeats. The recombinant vector can further comprise an AAV5 Rep-encoding nucleic acid. The vector encapsidated in the particle can further comprise an exogenous nucleic acid inserted between the inverted terminal repeats. AAV5 Rep confers targeted integration and efficient replication, thus production of recombinant

AAV5, comprising AAV5 Rep, yields more particles than production of recombinant AAV2. Since AAV5 is more efficient at replicating and packaging its genome, the exogenous nucleic acid inserted, or in the AAV5 capsids of the present invention, between the inverted terminal repeats can be packaged in the AAV1, 2, 3, 4, or 6 capsids to achieve the specific tissue tropism conferred by the capsid proteins.

The invention further contemplates chimeric recombinant ITRs that contains a rep binding site and a TRS site recognized by that Rep protein. By "Rep protein" is meant all four of the Rep proteins, Rep 40, Rep 78, Rep 52, Rep 68. Alternatively, "Rep protein" could be one or more of the Rep proteins described herein. One example of a chimeric ITR would consist of an AAV5 D region (SEQ ID NO: 23), an AAV5 TRS site (SEQ ID NO: 21), an AAV2 hairpin and an AAV2 binding site. Another example would be an AAV5 D region, an AAV5 TRS site, an AAV3 hairpin and an AAV3 binding site. In these chimeric ITRs, the D region can be from AAV1, 2, 3, 4, 5 or 6. The hairpin can be derived from AAV 1,2 3, 4, 5, 6. The binding site can be derived from any of AAV1, 2, 3, 4, 5 or 6. Preferably, the D region and the TRS are from the same serotype.

The chimeric ITRs can be combined with AAV5 Rep protein and any of the AAV serotype capsids to obtain recombinant virion. For example, recombinant virion can be produced by an AAV5 D region, an AAV5 TRS site, an AAV2 hairpin, an AAV2 binding site, AAV5 Rep protein and AAV1 capsid. This recombinant virion would possess the cellular tropism conferred by the AAV1 capsid protein and would possess the efficient replication conferred by the AAV5 Rep.

Other examples of the ITR, Rep protein and Capsids that will produce recombinant virus are provided in the list below:

5ITR + 5Rep + 5Cap=virus
5ITR + 5Rep + 1Cap=virus

5ITR + 5Rep + 2Cap=virus

5ITR + 5Rep + 3Cap=virus

5ITR + 5Rep + 4Cap=virus

5ITR + 5Rep + 6Cap=virus

1ITR + 1Rep + 5Cap=virus

2ITR + 2Rep + 5Cap=virus

3ITR + 3Rep + 5Cap=virus

4ITR + 4Rep + 5Cap=virus

6ITR + 6Rep + 5Cap=virus

In any of the constructs described herein, inclusion of a promoter is preferred. As used in the constructs herein, unless otherwise specified, Cap (capsid) refers to any of AAV5 VP1, AAV5 VP2, AAV5 VP3, combinations thereof, functional fragments of any of VP1, VP2 or VP3, or chimeric capsids as described herein. The ITRs of the constructs described herein, can be chimeric recombinant ITRs as described elsewhere in the application.

Conjugates of recombinant or wild-type AAV5 virions and nucleic acids or proteins can be used to deliver those molecules to a cell. For example, the purified AAV5 can be used as a vehicle for delivering DNA bound to the exterior of the virus. Examples of this are to conjugate the DNA to the virion by a bridge using poly-L-lysine or other charged molecule. Also contemplated are virosomes that contain AAV5 structural proteins (AAV5 capsid proteins), lipids such as DOTAP, and nucleic acids that are complexed via charge interaction to introduce DNA into cells.

Also provided by this invention are conjugates that utilize theAAV5 capsid or a unique region of the AAV5 capsid protein (e.g. VP1, VP2 or VP3 or combinations thereof) to introduce DNA into cells. For example, the type 5 VP3 protein or fragment thereof, can be conjugated to a DNA on a plasmid that is conjugated to a lipid. Cells can be infected using the targeting ability of the VP3 capsid protein to achieve the

desired tissue tropism, specific to AAV5. Type 5 VP1 and VP2 proteins can also be utilized to introduce DNA or other molecules into cells. By further incorporating the Rep protein and the AAV TRS into the DNA-containing conjugate, cells can be transduced and targeted integration can be achieved. For example, if AAV5 specific targeted integration is desired, a conjugate composed of the AAV5 VP3 capsid, AAV5 rep or a fragment of AAV5 rep, AAV5 TRS, the rep binding site, the heterologous DNA of interest, and a lipid, can be utilized to achieve AAV5 specific tropism and AAV5 specific targeted integration in the genome.

Further provided by this invention are chimeric viruses where AAV5 can be combined with herpes virus, baculovirus or other viruses to achieve a desired tropism associated with another virus. For example, the AAV5 ITRs could be inserted in the herpes virus and cells could be infected. Post-infection, the ITRs of AAV5 could be acted on by AAV5 rep provided in the system or in a separate vehicle to rescue AAV5 from the genome. Therefore, the cellular tropism of the herpes simplex virus can be combined with AAV5 rep mediated targeted integration. Other viruses that could be utilized to construct chimeric viruses include, lentivirus, retrovirus, pseudotyped retroviral vectors, and adenoviral vectors.

The present invention further provides isolated nucleic acids of AAV5. For example, provided is an isolated nucleic acid comprising the nucleotide sequence set forth in SEQ ID NO:1 (AAV5 genome). This nucleic acid, or portions thereof, can be inserted into vectors, such as plasmids, yeast artificial chromosomes, or other viral vector (particle), if desired, by standard cloning methods. The present invention also provides an isolated nucleic acid consisting essentially of the nucleotide sequence set forth in SEQ ID NO:1. The nucleotides of SEQ ID NO:1 can have minor modifications and still be contemplated by the present invention. For example, modifications that do not alter the amino acid encoded by any given codon (such as by modification of the third, "wobble," position in a codon) can readily be made, and such alterations are known in the art. Furthermore, modifications that cause a resulting neutral (conserved)

amino acid substitution of a similar amino acid can be made in a coding region of the genome. Additionally, modifications as described herein for the AAV5 components, such as the ITRs, the p5 promoter, etc. are contemplated in this invention. Furthermore, modifications to regions of SEQ ID NO:1 other than in the ITR, TRS Rep binding site and hairpin are likely to be tolerated without serious impact on the function of the nucleic acid as a recombinant vector.

As used herein, the term "isolated" refers to a nucleic acid separated or significantly free from at least some of the other components of the naturally occurring organism, for example, the cell structural components or viral components commonly found associated with nucleic acids in the environment of the virus and/or other nucleic acids. The isolation of the native nucleic acids can be accomplished, for example, by techniques such as cell lysis followed by phenol plus chloroform extraction, followed by ethanol precipitation of the nucleic acids. The nucleic acids of this invention can be isolated from cells according to any of many methods well known in the art.

As used herein, the term "nucleic acid" refers to single-or multiple stranded molecules which may be DNA or RNA, or any combination thereof, including modifications to those nucleic acids. The nucleic acid may represent a coding strand or its complement, or any combination thereof. Nucleic acids may be identical in sequence to the sequences which are naturally occurring for any of the novel genes discussed herein or may include alternative codons which encode the same amino acid as those provided herein, including that which is found in the naturally occurring sequence. These nucleic acids can also be modified from their typical structure. Such modifications include, but are not limited to, methylated nucleic acids, the substitution of a non-bridging oxygen on the phosphate residue with either a sulfur (yielding phosphorothioate deoxynucleotides), selenium (yielding phosphorselenoate deoxynucleotides), or methyl groups (yielding methylphosphonate deoxynucleotides).

The present invention additionally provides an isolated nucleic acid that selectively hybridizes with any nucleic acid disclosed herein, including the entire AAV5 genome and any unique fragment thereof, including the Rep and capsid encoding sequences (e.g. SEQ ID NOS: 1, 7, 8, 9, 10, 11, 13, 15, 16, 17, 18, 19, 20, 21, 22 and 23). Specifically, the nucleic acid can selectively or specifically hybridize to an isolated nucleic acid consisting of the nucleotide sequence set forth in SEQ ID NO:1 (AAV5 genome). The present invention further provides an isolated nucleic acid that selectively or specifically hybridizes with an isolated nucleic acid comprising the nucleotide sequence set forth in SEQ ID NO:1 (AAV5 genome). By "selectively hybridizes" as used herein is meant a nucleic acid that hybridizes to one of the disclosed nucleic acids under sufficient stringency conditions without significant hybridization to a nucleic acid encoding an unrelated protein, and particularly, without detectably hybridizing to nucleic acids of AAV2. Thus, a nucleic acid that selectively hybridizes with a nucleic acid of the present invention will not selectively hybridize under stringent conditions with a nucleic acid encoding a different protein or the corresponding protein from a different serotype of the virus, and vice versa. A "specifically hybridizing" nucleic acid is one that hybridizes under stringent conditions to only a nucleic acid found in AAV5. Therefore, nucleic acids for use, for example, as primers and probes to detect or amplify the target nucleic acids are contemplated herein. Nucleic acid fragments that selectively hybridize to any given nucleic acid can be used, *e.g.*, as primers and or probes for further hybridization or for amplification methods (*e.g.*, polymerase chain reaction (PCR), ligase chain reaction (LCR)). Additionally, for example, a primer or probe can be designed that selectively hybridizes with both AAV5 and a gene of interest carried within the AAV5 vector (*i.e.*, a chimeric nucleic acid).

Stringency of hybridization is controlled by both temperature and salt concentration of either or both of the hybridization and washing steps. Typically, the stringency of hybridization to achieve selective hybridization involves hybridization in high ionic strength solution (6X SSC or 6X SSPE) at a temperature that is about 12-

25°C below the T_m (the melting temperature at which half of the molecules dissociate from their hybridization partners) followed by washing at a combination of temperature and salt concentration chosen so that the washing temperature is about 5°C to 20°C below the T_m. The temperature and salt conditions are readily determined empirically in preliminary experiments in which samples of reference DNA immobilized on filters are hybridized to a labeled nucleic acid of interest and then washed under conditions of different stringencies. Hybridization temperatures are typically higher for DNA-RNA and RNA-RNA hybridizations. The washing temperatures can be used as described above to achieve selective stringency, as is known in the art. (Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989; Kunkel et al. *Methods Enzymol.* 1987:154:367, 1987). A preferable stringent hybridization condition for a DNA:DNA hybridization can be at about 68°C (in aqueous solution) in 6X SSC or 6X SSPE followed by washing at 68°C. Stringency of hybridization and washing, if desired, can be reduced accordingly as the degree of complementarity desired is decreased, and further, depending upon the G-C or A-T richness of any area wherein variability is searched for. Likewise, stringency of hybridization and washing, if desired, can be increased accordingly as homology desired is increased, and further, depending upon the G-C or A-T richness of any area wherein high homology is desired, all as known in the art.

A nucleic acid that selectively hybridizes to any portion of the AAV5 genome is contemplated herein. Therefore, a nucleic acid that selectively hybridizes to AAV5 can be of longer length than the AAV5 genome, it can be about the same length as the AAV5 genome or it can be shorter than the AAV5 genome. The length of the nucleic acid is limited on the shorter end of the size range only by its specificity for hybridization to AAV5, *i.e.*, once it is too short, typically less than about 5 to 7 nucleotides in length, it will no longer bind specifically to AAV5, but rather will hybridize to numerous background nucleic acids. Additionally contemplated by this invention is a nucleic acid that has a portion that specifically hybridizes to AAV5 and a portion that specifically hybridizes to a gene of interest inserted within AAV5.

The present invention further provides an isolated nucleic acid encoding an adeno-associated virus 5 Rep protein. The AAV5 Rep proteins are encoded by open reading frame (ORF) 1 of the AAV5 genome. Examples of the AAV5 Rep genes are shown in the nucleic acid set forth in SEQ ID NO:1, and include nucleic acids consisting essentially of the nucleotide sequences set forth in SEQ ID NOS:10 (Rep52), 11 (Rep78), 13 (Rep40), and 15 (Rep68), and nucleic acids comprising the nucleotide sequences set forth in SEQ ID NOS:10 , 11, 13, and 15. However, the present invention contemplates that the Rep nucleic acid can include any one, two, three, or four of the four Rep proteins, in any order, in such a nucleic acid. Furthermore, minor modifications are contemplated in the nucleic acid, such as silent mutations in the coding sequences, mutations that make neutral or conservative changes in the encoded amino acid sequence, and mutations in regulatory regions that do not disrupt the expression of the gene. Examples of other minor modifications are known in the art. Further modifications can be made in the nucleic acid, such as to disrupt or alter expression of one or more of the Rep proteins in order to, for example, determine the effect of such a disruption; such as to mutate one or more of the Rep proteins to determine the resulting effect, etc. However, in general, a modified nucleic acid encoding a Rep protein will have at least about 85%, about 90%, about 93%, about 95%, about 98% or 100% homology to the Rep nucleic sequences described herein e.g., SEQ ID NOS: 10, 11, 13 and 15, and the Rep polypeptide encoded therein will have overall about 93%, about 95%, about 98%, about 99% or 100% homology with the amino acid sequence described herein, e.g., SEQ ID NOS:2 , 3, 12 and 14. Percent homology is determined by the techniques described herein.

The present invention also provides an isolated nucleic acid that selectively or specifically hybridizes with a nucleic acid consisting essentially of the nucleotide sequence set forth in SEQ ID NOS:10, 11, 13 and 15, and an isolated nucleic acid that selectively hybridizes with a nucleic acid comprising the nucleotide sequence set forth in SEQ ID NOS:10, 11, 13 and 15. "Selectively hybridizing" and “stringency of hybridization” is defined elsewhere herein.

As described above, the present invention provides the nucleic acid encoding a Rep 40 protein and, in particular an isolated nucleic acid comprising the nucleotide sequence set forth in SEQ ID NO: 13, an isolated nucleic acid consisting essentially of the nucleotide sequence set forth in SEQ ID NO: 13, and a nucleic acid encoding the adeno-associated virus 5 protein having the amino acid sequence set forth in SEQ ID NO: 12. The present invention also provides the nucleic acid encoding a Rep 52 protein, and in particular an isolated nucleic acid comprising the nucleotide sequence set forth in SEQ ID NO:10, an isolated nucleic acid consisting essentially of the nucleotide sequence set forth in SEQ ID NO:10, and a nucleic acid encoding the adeno-associated virus 5 Rep protein having the amino acid sequence set forth in SEQ ID NO:2. The present invention further provides the nucleic acid encoding a Rep 68 protein and, in particular an isolated nucleic acid comprising the nucleotide sequence set forth in SEQ ID NO: 15, an isolated nucleic acid consisting essentially of the nucleotide sequence set forth in SEQ ID NO: 15, and a nucleic acid encoding the adeno-associated virus 5 protein having the amino acid sequence set forth in SEQ ID NO: 14. And, further, the present invention provides the nucleic acid encoding a Rep 78 protein, and in particular an isolated nucleic acid comprising the nucleotide sequence set forth in SEQ ID NO:11, an isolated nucleic acid consisting essentially of the nucleotide sequence set forth in SEQ ID NO:11, and a nucleic acid encoding the adeno-associated virus 5 Rep protein having the amino acid sequence set forth in SEQ ID NO:3. As described elsewhere herein, these nucleic acids can have minor modifications, including silent nucleotide substitutions, mutations causing conservative amino acid substitutions in the encoded proteins, and mutations in control regions that do not or minimally affect the encoded amino acid sequence.

The present invention further provides a nucleic acid encoding the entire AAV5 Capsid polypeptide. Furthermore, the present invention provides a nucleic acid encoding each of the three AAV5 coat proteins, VP1, VP2, and VP3. Thus, the present invention provides a nucleic acid encoding AAV5 VP1, a nucleic acid encoding AAV5 VP2, and a nucleic acid encoding AAV5 VP3. Thus, the present invention provides a

nucleic acid encoding the amino acid sequence set forth in SEQ ID NO:4 (VP1); a nucleic acid encoding the amino acid sequence set forth in SEQ ID NO:5 (VP2), and a nucleic acid encoding the amino acid sequence set forth in SEQ ID NO:6 (VP3). The present invention also specifically provides a nucleic acid comprising SEQ ID NO:7 (VP1 gene); a nucleic acid comprising SEQ ID NO:8 (VP2 gene); and a nucleic acid comprising SEQ ID NO:9 (VP3 gene). The present invention also specifically provides a nucleic acid consisting essentially of SEQ ID NO:7 (VP1 gene), a nucleic acid consisting essentially of SEQ ID NO:8 (VP2 gene), and a nucleic acid consisting essentially of SEQ ID NO:9 (VP3 gene). Minor modifications in the nucleotide sequences encoding the capsid, or coat, proteins are contemplated, as described above for other AAV5 nucleic acids. However, in general, a modified nucleic acid encoding a capsid protein will have at least about 85%, about 90%, about 93%, about 95%, about 98% or 100% homology to the capsid nucleic sequences described herein e.g., SEQ ID NOS: 7, 8, and 9, and the capsid polypeptide encoded therein will have overall about 93%, about 95%, about 98%, about 99% or 100% homology with the amino acid sequence described herein, e.g., SEQ ID NOS:4, 5, and 6. Nucleic acids that selectively hybridize with the nucleic acids of SEQ ID NOS:7,8 and 9 under the conditions described above are also provided.

The present invention also provides a cell containing one or more of the herein described nucleic acids, such as the AAV5 genome, AAV5 ORF1 and ORF2, each AAV5 Rep protein gene, or each AAV5 capsid protein gene. Such a cell can be any desired cell and can be selected based upon the use intended. For example, cells can include bacterial cells, yeast cells, insect cells, human HeLa cells and simian Cos cells as well as other human and mammalian cells and cell lines. Primary cultures as well as established cultures and cell lines can be used. Nucleic acids of the present invention can be delivered into cells by any selected means, in particular depending upon the target cells. Many delivery means are well-known in the art. For example, electroporation, calcium phosphate precipitation, microinjection, cationic or anionic liposomes, and liposomes in combination with a nuclear localization signal peptide for

delivery to the nucleus can be utilized, as is known in the art. Additionally, if the nucleic acids are in a viral particle, the cells can simply be transduced with the virion by standard means known in the art for AAV transduction. Small amounts of the recombinant AAV5 virus can be made to infect cells and produce more of itself.

The invention provides purified AAV5 polypeptides. The term "polypeptide" as used herein refers to a polymer of amino acids and includes full-length proteins and fragments thereof. Thus, "protein," polypeptide," and "peptide" are often used interchangeably herein. Substitutions can be selected by known parameters to be neutral (*see, e.g.,* Robinson WE Jr, and Mitchell WM., AIDS 4:S151-S162 (1990)). As will be appreciated by those skilled in the art, the invention also includes those polypeptides having slight variations in amino acid sequences or other properties. Such variations may arise naturally as allelic variations (*e.g.,* due to genetic polymorphism) or may be produced by human intervention (*e.g.,* by mutagenesis of cloned DNA sequences), such as induced point, deletion, insertion and substitution mutants. Minor changes in amino acid sequence are generally preferred, such as conservative amino acid replacements, small internal deletions or insertions, and additions or deletions at the ends of the molecules. Substitutions may be designed based on, for example, the model of Dayhoff, *et al.* (in *Atlas of Protein Sequence and Structure 1978*, Nat'l Biomed. Res. Found., Washington, D.C.). These modifications can result in changes in the amino acid sequence, provide silent mutations, modify a restriction site, or provide other specific mutations. The location of any modifications to the polypeptide will often determine its impact on function. Particularly, alterations in regions non-essential to protein function will be tolerated with fewer effects on function. Elsewhere in the application regions of the AAV5 proteins are described to provide guidance as to where substitutions, additions or deletions can be made to minimize the likelihood of disturbing the function of the variant.

A polypeptide of the present invention can be readily obtained by any of several means. For example, the polypeptide of interest can be synthesized chemically by

standard methods. Additionally, the coding regions of the genes can be recombinantly expressed and the resulting polypeptide isolated by standard methods. Furthermore, an antibody specific for the resulting polypeptide can be raised by standard methods (see, *e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1988), and the protein can be isolated from a cell expressing the nucleic acid encoding the polypeptide by selective hybridization with the antibody. This protein can be purified to the extent desired by standard methods of protein purification (see, *e.g.*, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989).

Typically, to be unique, a polypeptide fragment of the present invention will be at least about 5 amino acids in length; however, unique fragments can be 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or more amino acids in length. A unique polypeptide will typically comprise such a unique fragment; however, a unique polypeptide can also be determined by its overall homology. A unique polypeptide can be 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or more amino acids in length. Uniqueness of a polypeptide fragment can readily be determined by standard methods such as searches of computer databases of known peptide or nucleic acid sequences or by hybridization studies to the nucleic acid encoding the protein or to the protein itself, as known in the art. The uniqueness of a polypeptide fragment can also be determined immunologically as well as functionally. Uniqueness can be simply determined in an amino acid-by-amino acid comparison of the polypeptides.

An antigenic or immunoreactive fragment of this invention is typically an amino acid sequence of at least about 5 consecutive amino acids, and it can be derived from the AAV5 polypeptide amino acid sequence. An antigenic AAV5 fragment is any fragment unique to the AAV5 protein, as described herein, against which an AAV5-specific antibody can be raised, by standard methods. Thus, the resulting antibody-antigen reaction should be specific for AAV5.

The present invention provides an isolated AAV5 Rep protein. An AAV5 Rep polypeptide is encoded by ORF1 of AAV5. The present invention also provides each individual AAV5 Rep protein. Thus the present invention provides AAV5 Rep 40 (e.g., SEQ ID NO: 12), or a unique fragment thereof. The present invention provides AAV5 Rep 52 (e.g., SEQ ID NO: 2), or a unique fragment thereof. The present invention provides AAV5 Rep 68 (e.g., SEQ ID NO: 14), or a unique fragment thereof. The present invention provides an example of AAV5 Rep 78 (e.g., SEQ ID NO: 3), or a unique fragment thereof. By "unique fragment thereof" is meant any smaller polypeptide fragment encoded by an AAV5 rep gene that is of sufficient length to be found only in the Rep polypeptide. Substitutions and modifications of the amino acid sequence can be made as described above and, further, can include protein processing modifications, such as glycosylation, to the polypeptide.

The present invention further provides an AAV5 Capsid polypeptide or a unique fragment thereof. AAV5 capsid polypeptide is encoded by ORF 2 of AAV5. The present invention further provides the individual AAV5 capsid proteins, VP1, VP2 and VP3 or unique fragments thereof. Thus, the present invention provides an isolated polypeptide having the amino acid sequence set forth in SEQ ID NO:4 (VP1). The present invention additionally provides an isolated polypeptide having the amino acid sequence set forth in SEQ ID NO:5 (VP2). The present invention also provides an isolated polypeptide having the amino acid sequence set forth in SEQ ID NO:6 (VP3). By "unique fragment thereof" is meant any smaller polypeptide fragment encoded by any AAV5 capsid gene that is of sufficient length to be found only in the AAV5 capsid protein. Substitutions and modifications of the amino acid sequence can be made as described above and, further, can include protein processing modifications, such as glycosylation, to the polypeptide. However, an AAV5 Capsid polypeptide including all three coat proteins will have greater than about 56% overall homology to the polypeptide encoded by the nucleotides set forth in SEQ ID NOS:4,5 or 6. The protein can have about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, 93%, 95%, 97% or even 100% homology to the amino acid sequence encoded by the

nucleotides set forth in SEQ ID NOS:4,5 or 6. An AAV5 VP1 polypeptide can have at least about 58%, about 60%, about 70%, about 80%, about 90%, 93%, 95%, 97% or about 100% homology to the amino acid sequence set forth in SEQ ID NO:4. An AAV5 VP2 polypeptide can have at least about 58%, about 60%, about 70%, about 80%, about 90%, 93%, 95%, 97% or about 100% homology to the amino acid sequence set forth in SEQ ID NO:5. An AAV5 VP3 polypeptide can have at least about 60%, about 70%, about 80%, about 90%, 93%, 95%, 97% or about 100% homology to the amino acid sequence set forth in SEQ ID NO:6.

The present invention further provides an isolated antibody that specifically binds an AAV5 Rep protein or a unique epitope thereof. Also provided are isolated antibodies that specifically bind the AAV5 Rep 52 protein, the AAV5 Rep 40 protein, the AAV5 Rep 68 protein and the AAV5 Rep 78 protein having the amino acid sequences set forth in SEQ ID NO:2, SEQ ID NO: 12, SEQ ID NO: 14 and SEQ ID NO: 3, respectively or that specifically binds a unique fragment thereof. Clearly, any given antibody can recognize and bind one of a number of possible epitopes present in the polypeptide; thus only a unique portion of a polypeptide (having the epitope) may need to be present in an assay to determine if the antibody specifically binds the polypeptide.

The present invention additionally provides an isolated antibody that specifically binds any of the adeno-associated virus 5 Capsid proteins (VP1, VP2 or VP3), a unique epitope thereof, or the polypeptide comprising all three AAV5 coat proteins. Also provided is an isolated antibody that specifically binds the AAV5 capsid protein having the amino acid sequence set forth in SEQ ID NO:4 (VP1), or that specifically binds a unique fragment thereof. The present invention further provides an isolated antibody that specifically binds the AAV5 Capsid protein having the amino acid sequence set forth in SEQ ID NO:5 (VP2), or that specifically binds a unique fragment thereof. The invention additionally provides an isolated antibody that specifically binds the AAV5 Capsid protein having the amino acid sequence set forth in

SEQ ID NO:6 (VP3), or that specifically binds a unique fragment thereof. Again, any given antibody can recognize and bind one of a number of possible epitopes present in the polypeptide; thus only a unique portion of a polypeptide (having the epitope) may need to be present in an assay to determine if the antibody specifically binds the polypeptide.

The antibody can be a component of a composition that comprises an antibody that specifically binds the AAV5 protein. The composition can further comprise, *e.g.*, serum, serum-free medium, or a pharmaceutically acceptable carrier such as physiological saline, etc..

By "an antibody that specifically binds" an AAV5 polypeptide or protein is meant an antibody that selectively binds to an epitope on any portion of the AAV5 peptide such that the antibody binds specifically to the corresponding AAV5 polypeptide without significant background. Specific binding by an antibody further means that the antibody can be used to selectively remove the target polypeptide from a sample comprising the polypeptide or and can readily be determined by radioimmunoassay (RIA), bioassay, or enzyme-linked immunosorbant (ELISA) technology. An ELISA method effective for the detection of the specific antibody-antigen binding can, for example, be as follows: (1) bind the antibody to a substrate; (2) contact the bound antibody with a sample containing the antigen; (3) contact the above with a secondary antibody bound to a detectable moiety (e.g., horseradish peroxidase enzyme or alkaline phosphatase enzyme); (4) contact the above with the substrate for the enzyme; (5) contact the above with a color reagent; (6) observe the color change.

An antibody can include antibody fragments such as Fab fragments which retain the binding activity. Antibodies can be made as described in, *e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1988). Briefly, purified antigen can be injected into an animal in

an amount and in intervals sufficient to elicit an immune response. Antibodies can either be purified directly, or spleen cells can be obtained from the animal. The cells are then fused with an immortal cell line and screened for antibody secretion. Individual hybridomas are then propagated as individual clones serving as a source for a particular monoclonal antibody.

The present invention additionally provides a method of screening a cell for infectivity by AAV5 comprising contacting the cell with AAV5 and detecting the presence of AAV5 in the cells. AAV5 particles can be detected using any standard physical or biochemical methods. For example, physical methods that can be used for this detection include DNA based methods such as 1) polymerase chain reaction (PCR) for viral DNA or RNA or 2) direct hybridization with labeled probes, and immunological methods such as by 3) antibody directed against the viral structural or non- structural proteins. Catalytic methods of viral detection include, but are not limited to, detection of site and strand specific DNA nicking activity of Rep proteins or replication of an AAV origin- containing substrate. Reporter genes can also be utilized to detect cells that transduct AAV-5. For example, β-gal, green flourescent protein or luciferase can be inserted into a recombinant AAV-5. The cell can then be contacted with the recombinant AAV-5, either *in vitro* or *in vivo* and a colorimetric assay could detect a color change in the cells that would indicate transduction of AAV-5 in the cell. Additional detection methods are outlined in Fields, *Virology*, Raven Press, New York, New York. 1996.

For screening a cell for infectivity by AAV5, wherein the presence of AAV5 in the cells is determined by nucleic acid hybridization methods, a nucleic acid probe for such detection can comprise, for example, a unique fragment of any of the AAV5 nucleic acids provided herein. The uniqueness of any nucleic acid probe can readily be determined as described herein. Additionally, the presence of AAV5 in cells can be determined by flourescence, antibodies to gene products, focus forming assays, plaque lifts, Western blots and chromogenic assays. The nucleic acid can be, for example, the

nucleic acid whose nucleotide sequence is set forth in SEQ ID NO: 1,7, 8, 9, 10, 11, 13, 15, 16, 17, 18, 19, 20, 21, 22, 23 or a unique fragment thereof.

The present invention includes a method of determining the suitability of an AAV5 vector for administration to a subject comprising administering to an antibody-containing sample from the subject an antigenic fragment of an isolated AAV5 Rep or Capsid protein, and detecting neutralizing antibody-antigen reaction in the sample, the presence of a neutralizing reaction indicating the AAV5 vector may be unsuitable for use in the subject. The present method of determining the suitability of an AAV5 vector for administration to a subject can comprise contacting an antibody-containing sample from the subject with a unique antigenic or immunogenic fragment of an AAV5 Rep protein (e.g. Rep 40, Rep 52, Rep 68, Rep 78) and detecting an antibody-antigen reaction in the sample, the presence of a reaction indicating the AAV5 vector to be unsuitable for use in the subject. The AAV5 Rep proteins are provided herein, and their antigenic fragments are routinely determined. The AAV5 capsid protein can be used to select an antigenic or immunogenic fragment, for example from the amino acid sequence set forth in SEQ ID NO:4 (VP1), the amino acid sequence set forth in SEQ ID NO: 5 (VP2) or the amino acid sequence set forth in SEQ ID NO:6 (VP3). Alternatively, or additionally, an antigenic or immunogenic fragment of an isolated AAV5 Rep protein can be utilized in this determination method. The AAV5 Rep protein from which an antigenic fragment is selected can have the amino acid sequence encoded by the nucleic acid set forth in SEQ ID NO:1, the amino acid sequence set forth in SEQ ID NO:2, or the amino acid sequence set forth in SEQ ID NO:3, the amino acid sequence set forth in SEQ ID NO: 12, or the amino acid sequence set forth in SEQ ID NO:14.

The AAV5 polypeptide fragments can be analyzed to determine their antigenicity, immunogenicity and/or specificity. Briefly, various concentrations of a putative immunogenically specific fragment are prepared and administered to a subject and the immunological response (e.g., the production of antibodies or cell mediated

immunity) of an animal to each concentration is determined. The amounts of antigen administered depend on the subject, e.g. a human, rabbit or a guinea pig, the condition of the subject, the size of the subject, etc. Thereafter an animal so inoculated with the antigen can be exposed to the AAV5 viral particle or AAV5 protein to test the immunoreactivity or the antigenicity of the specific immunogenic fragment. The specificity of a putative antigenic or immunogenic fragment can be ascertained by testing sera, other fluids or lymphocytes from the inoculated animal for cross reactivity with other closely related viruses, such as AAV1, AAV2, AAV3, AAV4 and AAV5.

The hemagglutination assay can also be used to rapidly identify and detect AAV5 viral particles. Detection of hemagglutination activity correlates with infectivity and can be used to titer the virus. This assay could also be used to identify antibodies in a patients serum which might interact with the virus. Hemagglutination has been shown to correlate with infectivity and therefore hemagglutination may be a useful assay for identify cellular receptors for AAV5.

By the "suitability of an AAV5 vector for administration to a subject" is meant a determination of whether the AAV5 vector will elicit a neutralizing immune response upon administration to a particular subject. A vector that does not elicit a significant immune response is a potentially suitable vector, whereas a vector that elicits a significant, neutralizing immune response (e.g. at least 90%) is thus likely to be unsuitable for use in that subject. Significance of any detectable immune response is a standard parameter understood by the skilled artisan in the field. For example, one can incubate the subject's serum with the virus, then determine whether that virus retains its ability to transduce cells in culture. If such virus cannot transduce cells in culture, the vector likely has elicited a significant immune response.

Alternatively, or additionally, one skilled in the art could determine whether or not AAV5 administration would be suitable for a particular cell type of a subject. For example, the artisan could culture muscle cells *in vitro* and transduce the cells with

AAV5 in the presence or absence of the subject's serum. If there is a reduction in transduction efficiency, this could indicate the presence of a neutralizing antibody or other factors that may inhibit transduction. Normally, greater than 90% inhibition would have to be observed in order to rule out the use of AAV-5 as a vector. However, this limitation could be overcome by treating the subject with an immunosuppressant that could block the factors inhibiting transduction.

As will be recognized by those skilled in the art, numerous types of immunoassays are available for use in the present invention to detect binding between an antibody and an AAV5 polypeptide of this invention. For instance, direct and indirect binding assays, competitive assays, sandwich assays, and the like, as are generally described in, *e.g.*, U.S. Pat. Nos. 4,642,285; 4,376,110; 4,016,043; 3,879,262; 3,852,157; 3,850,752; 3,839,153; 3,791,932; and Harlow and Lane, *Antibodies, A Laboratory Manual*, Cold Spring Harbor Publications, N.Y. (1988). For example, enzyme immunoassays such as immunofluorescence assays (IFA), enzyme linked immunosorbent assays (ELISA) and immunoblotting can be readily adapted to accomplish the detection of the antibody. An ELISA method effective for the detection of the antibody bound to the antigen can, for example, be as follows: (1) bind the antigen to a substrate; (2) contact the bound antigen with a fluid or tissue sample containing the antibody; (3) contact the above with a secondary antibody specific for the antigen and bound to a detectable moiety (e.g., horseradish peroxidase enzyme or alkaline phosphatase enzyme); (4) contact the above with the substrate for the enzyme; (5) contact the above with a color reagent; (6) observe color change.

The antibody-containing sample of this method can comprise any biological sample which would contain the antibody or a cell containing the antibody, such as blood, plasma, serum, bone marrow, saliva and urine.

The present invention also provides a method of producing the AAV5 virus by transducing a cell with the nucleic acid encoding the virus.

The present method further provides a method of delivering an exogenous (heterologous) nucleic acid to a cell comprising administering to the cell an AAV5 particle containing a vector comprising the nucleic acid inserted between a pair of AAV inverted terminal repeats, thereby delivering the nucleic acid to the cell.

The AAV ITRs in the vector for the herein described delivery methods can be AAV5 ITRs (SEQ ID NOS: 19 and 20). Furthermore, the AAV ITRs in the vector for the herein described nucleic acid delivery methods can also comprise AAV1, AAV2 , AAV3, AAV4, or AAV6 inverted terminal repeats.

The present invention also includes a method of delivering a heterologous nucleic acid to a subject comprising administering to a cell from the subject an AAV5 particle containing a vector comprising the nucleic acid inserted between a pair of AAV inverted terminal repeats, and returning the cell to the subject, thereby delivering the nucleic acid to the subject. The AAV ITRs can be any AAV ITRs, including AAV5 ITRs and AAV2 ITRs. For example, in an *ex vivo* administration, cells are isolated from a subject by standard means according to the cell type and placed in appropriate culture medium, again according to cell type (*see, e.g.*, ATCC catalog). Viral particles are then contacted with the cells as described above, and the virus is allowed to transduce the cells. Cells can then be transplanted back into the subject's body, again by means standard for the cell type and tissue (*e. g.*, in general, U.S. Patent No. 5,399,346; for neural cells, Dunnett, S.B. and Björklund, A., eds., *Transplantation: Neural Transplantation-A Practical Approach,* Oxford University Press, Oxford (1992)). If desired, prior to transplantation, the cells can be studied for degree of transduction by the virus, by known detection means and as described herein. Cells for *ex vivo* transduction followed by transplantation into a subject can be selected from those listed above, or can be any other selected cell. Preferably, a selected cell type is examined for its capability to be transfected by AAV5. Preferably, the selected cell will be a cell readily transduced with AAV5 particles; however, depending upon the application, even cells with relatively low transduction efficiencies can be useful,

particularly if the cell is from a tissue or organ in which even production of a small amount of the protein or antisense RNA encoded by the vector will be beneficial to the subject.

The present invention further provides a method of delivering a nucleic acid to a cell in a subject comprising administering to the subject an AAV5 particle containing a vector comprising the nucleic acid inserted between a pair of AAV inverted terminal repeats, thereby delivering the nucleic acid to a cell in the subject. Administration can be an *ex vivo* administration directly to a cell removed from a subject, such as any of the cells listed above, followed by replacement of the cell back into the subject, or administration can be *in vivo* administration to a cell in the subject. For *ex vivo* administration, cells are isolated from a subject by standard means according to the cell type and placed in appropriate culture medium, again according to cell type (*see, e.g.*, ATCC catalog). Viral particles are then contacted with the cells as described above, and the virus is allowed to transfect the cells. Cells can then be transplanted back into the subject's body, again by means standard for the cell type and tissue (*e. g.,* for neural cells, Dunnett, S.B. and Björklund, A., eds., *Transplantation: Neural Transplantation-A Practical Approach,* Oxford University Press, Oxford (1992)). If desired, prior to transplantation, the cells can be studied for degree of transfection by the virus, by known detection means and as described herein.

The present invention further provides a method of delivering a nucleic acid to a cell in a subject having neutralizing antibodies to AAV2 comprising administering to the subject an AAV5 particle containing a vector comprising the nucleic acid, thereby delivering the nucleic acid to a cell in the subject. A subject that has neutralizing antibodies to AAV2 can readily be determined by any of several known means, such as contacting AAV2 protein(s) with an antibody-containing sample, such as blood, from a subject and detecting an antigen-antibody reaction in the sample. Delivery of the AAV5 particle can be by either *ex vivo* or *in vivo* administration as herein described. Thus, a subject who might have an adverse immunogenic reaction to a vector

administered in an AAV2 viral particle can have a desired nucleic acid delivered using an AAV5 particle. This delivery system can be particularly useful for subjects who have received therapy utilizing AAV2 particles in the past and have developed antibodies to AAV2. An AAV5 regimen can now be substituted to deliver the desired nucleic acid.

In any of the methods of delivering heterologous nucleic acids to a cell or subject described herein, the AAV5-conjugated nucleic acid or AAV5 particle-conjugated nucleic acids described herein can be used.

In vivo administration to a human subject or an animal model can be by any of many standard means for administering viruses, depending upon the target organ, tissue or cell. Virus particles can be administered orally, parenterally (*e.g.*, intravenously), by intramuscular injection, by direct tissue or organ injection, by intraperitoneal injection, topically, transdermally, via aerosol delivery, via the mucosa or the like. Viral nucleic acids (non-encapsidated) can also be administered, *e.g.*, as a complex with cationic liposomes, or encapsulated in anionic liposomes. The present compositions can include various amounts of the selected viral particle or non-encapsidated viral nucleic acid in combination with a pharmaceutically acceptable carrier and, in addition, if desired, may include other medicinal agents, pharmaceutical agents, carriers, adjuvants, diluents, etc. Parental administration, if used, is generally characterized by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Dosages will depend upon the mode of administration, the disease or condition to be treated, and the individual subject's condition, but will be that dosage typical for and used in administration of other AAV vectors, such as AAV2 vectors. Often a single dose can be sufficient; however, the dose can be repeated if desirable.

Administration methods can be used to treat brain disorders such as Parkinson's disease, Alzheimer's disease, and demyelination disease. Other diseases that can be

treated by these methods include metabolic disorders such as , muscoloskeletal diseases, cardiovascular disease, cancer, and autoimmune disorders.

Administration of this recombinant AAV5 virion to the cell can be accomplished by any means, including simply contacting the particle, optionally contained in a desired liquid such as tissue culture medium, or a buffered saline solution, with the cells. The virion can be allowed to remain in contact with the cells for any desired length of time, and typically the virion is administered and allowed to remain indefinitely. For such *in vitro* methods, the virion can be administered to the cell by standard viral transduction methods, as known in the art and as exemplified herein. Titers of virus to administer can vary, particularly depending upon the cell type, but will be typical of that used for AAV transduction in general which is well known in the art. Additionally the titers used to transduce the particular cells in the present examples can be utilized.

The cells that can be transduced by the present recombinant AAV5 virion can include any desired cell, such as the following cells and cells derived from the following tissues, human as well as other mammalian tissues, such as primate, horse, sheep, goat, pig, dog, rat, and mouse: Adipocytes, Adenocyte, Adrenal cortex, Amnion, Aorta, Ascites, Astrocyte, Bladder, Bone, Bone marrow, Brain, Breast, Bronchus, Cardiac muscle, Cecum, Cervix, Chorion, Colon, Conjunctiva, Connective tissue, Cornea, Dermis, Duodenum, Endometrium, Endothelium, Endothelial cells, Epithelial tissue, Epithelial cells, Epidermis, Esophagus, Eye, Fascia, Fibroblasts, Foreskin, Gastric, Glial cells, Glioblast, Gonad, Hepatic cells, Histocyte, Ileum, Intestine, small Intestine, Jejunum, Keratinocytes, Kidney, Larynx, Leukocytes, Lipocyte, Liver, Lung, Lymph node, Lymphoblast, Lymphocytes, Macrophages, Mammary alveolar nodule, Mammary gland, Mastocyte, Maxilla, Melanocytes, Mesenchymal, Monocytes, Mouth, Myelin, Myoblasts Nervous tissue, Neuroblast, Neurons, Neuroglia, Osteoblasts, Osteogenic cells, Ovary, Palate, Pancreas, Papilloma, Peritoneum, Pituicytes, Pharynx, Placenta, Plasma cells, Pleura, Prostate, Rectum, Salivary gland, Skeletal muscle, Skin,

Smooth muscle, Somatic, Spleen, Squamous, Stomach, Submandibular gland, Submaxillary gland, Synoviocytes, Testis, Thymus, Thyroid, Trabeculae, Trachea, Turbinate, Umbilical cord, Ureter, and Uterus.

STATEMENT OF UTILITY

The present invention provides recombinant vectors based on AAV5. Such vectors may be useful for transducing erythroid progenitor cells or cells lacking heparin sulfate proteoglycans which is very inefficient with AAV2 based vectors. These vectors may also be useful for transducing cells with a nucleic acid of interest in order to produce cell lines that could be used to screen for agents that interact with the gene product of the nucleic acid of interest. In addition to transduction of other cell types, transduction of erythroid cells would be useful for the treatment of cancer and genetic diseases which can be corrected by bone marrow transplants using matched donors. Some examples of this type of treatment include, but are not limited to, the introduction of a therapeutic gene such as genes encoding interferons, interleukins, tumor necrosis factors, adenosine deaminase, cellular growth factors such as lymphokines, blood coagulation factors such as factor VIII and IX, cholesterol metabolism uptake and transport protein such as EpoE and LDL receptor, and antisense sequences to inhibit viral replication of, for example, hepatitis or HIV.

The present invention provides a vector comprising the AAV5 virus as well as AAV5 viral particles. While AAV5 is similar to AAV2, the two viruses are found herein to be physically and genetically distinct. These differences endow AAV5 with some unique advantages which better suit it as a vector for gene therapy.

Furthermore, as shown herein, AAV5 capsid protein is distinct from AAV2 capsid protein and exhibits different tissue tropism. AAV2 and AAV5 likely utilize distinct cellular receptors. AAV2 and AAV5 are serologically distinct and thus, in a gene therapy application, AAV5 would allow for transduction of a patient who already

possess neutralizing antibodies to AAV2 either as a result of natural immunological defense or from prior exposure to AAV2 vectors.

The present invention is more particularly described in the following examples which are intended as illustrative only since numerous modifications and variations therein will be apparent to those skilled in the art.

EXAMPLES

To understand the nature of AAV5 virus and to determine its usefulness as a vector for gene transfer, it was cloned and sequenced.

Cell culture and virus propagation

Cos and HeLa cells were maintained as monolayer cultures in D10 medium (Dulbecco's modified Eagle's medium containing 10% fetal calf serum, 100 μg/ml penicillin, 100 units/ml streptomycin and IX Fungizone as recommended by the manufacturer; (GIBCO, Gaithersburg, MD, USA) . All other cell types were grown under standard conditions which have been previously reported.

Virus was produced as previously described for AAV2 using the Beta galactosidase vector plasmid and a helper plasmid containing the AAV5 Rep and Cap genes (9). The helper plasmid was constructed in such a way to minimize any homologous sequence between the helper and vector plasmids. This step was taken to minimize the potential for wild-type (wt) particle formation by homologous recombination.

DNA Cloning and Sequencing and Analysis

In order to clone the genome of AAV5, infectious cell lysate was expanded in adherent cos cells and then suspension HeLa cells with the resulting viral particles isolated by CsCl isopynic gradient centrifugation. DNA dot blots of Aliquots of the

gradient fractions indicated that the highest concentration of viral genomes were contained in fractions with a refractive index of approx. 1.372. While the initial description of the virus did not determine the density of the particles, this value is similar to that of AAV2. Analysis of annealed virion derived DNA obtained from these fractions indicated a major species of 4.6 kb in length which upon restriction analysis gave bands similar in size to those previously reported. Additional restriction mapping indicated a unique BssHII site at one end of the viral genome. This site was used to clone the major fragment of the viral genome. Additional overlapping clones were isolated and the sequence determined. Two distinct open reading frames (ORF) were identified. Computer analysis indicated that the left-hand ORF is approx 60% similar to that of the Rep gene of AAV2. Of the 4 other reported AAV serotypes, all have greater than 90% similarity in this ORF. The right ORF of the viral capsid proteins is also approximately 60% homologous to the Capsid ORF of AAV2. As with other AAV serotypes reported, the divergence between AAV5 and AAV2 is clustered in multiple blocks. By using the published three dimensional structure of the canine parvovirus and computer aided sequence comparisons, a number of these divergent regions have been shown to be on the exterior of the virus and thus suggest an altered tissue tropism.

Within the p5 promoter, a number of the core transcriptional elements are conserved such as the tataa box and YY1 site around the transcriptional start site. However the YY1 site at -60 and the upstream E-Box elements are not detectable suggesting an alternative method of regulation or activation.

The inverted terminal repeats (ITRs) of the virus were cloned as a fragment from the right end of the genome. The resulting fragment was found to contain a number of sequence changes compared to AAV2. However, these changes were found to be complementary and did not affect the ability of this region to fold into a hairpin structure. Within the stem region of the hairpin two sequence elements have been found to be critical for the function of the ITRs as origins of viral replication. A repeat

motif of GAGC/T which serves as the recognition site of Rep and a GGTTGAG sequence downstream of the Rep binding site which is the position of Rep's site and strand specific cleavage reaction. This sequence is not conserved between AAV5 and the other cloned AAV's suggesting that the ITRs and Rep proteins of AAV5 cannot compliment the other known AAV's.

To test the cross complementarity of AAV2 ITR containing genome and AAV5 ITR containing genomes recombinant particles were packaged either using type 2 Rep and Cap or type 5 Rep and Cap expression plasmids as previously described. As shown in Fig. 2, viral particles were produced only when the respective expression plasmids were used to package the cognate ITRs. This result is distinct from that of other serotypes of AAV which have shown cross complementary in packaging.

This specificity of AAV5 Rep for AAV5 ITRs was confirmed using a terminal resolution assay which can identify the site within one ITR cleaved by the Rep protein. Incubation of the Type 5 Rep protein with a type 2 ITR did not produce any cleavage products. In contrast, addition of type 2 Rep cleaved the DNA at the expected site. However AAV5 Rep did produce cleavage products when incubated with a type 5 ITR. The site mapped to a region 21 bases from the Rep binding motif that is similar to AAV2 TRS. The site in AAV2 is CGGT TGAG (SEQ ID NO: 22) but in type 5 ITR is CGGT GTGA (SEQ ID NO: 21). The ability of AAV5 Rep to cleave at a different but similarly positioned site may result in integration of AAV5 at a distinct chromosomal locus compared to AAV2.

Recombinant virus produced using AAV5 Rep and Cap was obtained at a greater titer than type 2. For example, in a comparative study, virus was isolated from $8X10^7$ COS cells by CsCl banding and the distribution of the Beta galactosidase genomes across the gradient were determined by DNA dot blots of aliquots of gradient fractions. DNA dot blot titers indicated that AAV5 particles were produced at a 10-50 fold higher level than AAV2.

The sequence divergence in the capsid protein ORF implies that the tissue tropism of AAV2 and AAV5 would differ. To study the transduction efficiency of AAV5 and AAV2, a variety of cell lines were transduced with serial dilution's of the purified virus expressing the gene for nuclear localized Beta galactosidase activity. Approx. $2X10^4$ cells were exposed to virus in 1 ml of serum containing media for a period of 48-60 hrs. After this time the cells were fixed and stained for Beta-galactosidase activity with 5-Bromo-4-chloro- 3-indolyl-b-D- galactopyranoside (Xgal) (ICN Biochemicals). Biological titers were determined by counting the number of positive cells in the different dilutions using a calibrated microscope ocular then multiplying by the area of the well. Titers were determined by the average number of cells in a minimum of 10 fields/well. Transduction of cos, HeLa, and 293, and IB3 cells with a similar number of particles showed approximately 10 fold decrease in titer with AAV5 compared with AAV2. In contrast MCF7 cells showed a 50-100 fold difference in transduction efficiency. Furthermore, both vectors transduced NIH 3T3 cells relatively poorly.

A recent publication reported that heparin proteoglycans on the surface of cells are involved in viral transduction. Addition of soluble heparin has been shown to inhibit transduction by blocking viral binding. Since the transduction data suggested a difference in tissue tropism for AAV5 and AAV2, the sensitivity of AAV5 transduction to heparin was determined. At an MOI of 100, the addition of 20μg/ml of heparin had no effect on AAV5 transduction. In contrast this amount of heparin inhibited 90% of the AAV2 transduction. Even at an MOI of 1000, no inhibition of AAV5 transduction was detected. These data support the conclusions of the tissue tropism study, i.e. that AAV2 and AAV5 may utilize a distinct cell surface molecules and therefore the mechanism of uptake may differ as well.

AAV5 is a distinct virus within the dependovirus family based on sequence analysis, tissue tropism, and sensitivity to heparin. While elements of the P5 promoter are retained between AAV2-6 some elements are absent in AAV5 suggesting

alternative mechanism of regulation. The ITR and Rep ORF are distinct from those previously identified and fail to complement the packaging of AAV2 based genomes. The ITR of AAV5 contains a different TRS compared to other serotypes of AAV which is responsible for the lack of complementation of the ITRs. This unique TRS should also result in a different integration locus for AAV5 compared to that of AAV2. Furthermore the production of recombinant AAV5 particles using standard packaging systems is approx. 10-50 fold better than AAV2. The majority of the differences in the capsid proteins lies in regions which have been proposed to be on the exterior of the surface of the parvovirus. These changes are most likely responsible for the lack of cross reactive antibodies and altered tissue tropism compared to AAV2.

From the Rep ORF of AAV2, 4 proteins are produced; The p5 promoter (SEQ ID NO: 18) produces rep 68 (a spliced site mutant) and rep78 and the p19 promoter (SEQ ID NO: 16) produces rep 40 (a spliced site mutant) and rep 52. While these regions are not well conserved within the Rep ORF of AAV5 some splice acceptor and donor sites exist in approximately the same region as the AAV2 sites. These sites can be identified using standard computer analysis programs such as signal in the PCGENE program. Therefore the sequences of the Rep proteins can be routinely identified as in other AAV serotypes.

Hemagglutination assay

Hemagglutination activity was measured essentially as described previously (Chiorini et al 1997 J. Virol. Vol 71 6823-6833) Briefly 2 fold serial dilutions of virus in EDTA-buffered saline were mixed with an equal volume of 0.4% red blood cells in plastic U-bottom 96 well plates. The reaction was complete after a 2-h incubation at 8°C. Addition of purified AAV5 to a hemagglutination assay resulted in hemagglutination activity.

Transduction of airway epithelial cells

Primary airway epithilial cells were cultured and plated as previously described (Fasbender et al. J. Clin Invest. 1998 Jul 1; 102 (1): 184-93). Cells were transducted with an equivalent number of rAAV2 or rAAV5 particles containing a nuclear localized β-gal transgene with 50 particles of virus/cell (MOI 50) and continued in culture for 10 days. β-gal activity was determined following the procedure of (Chiorini et al. 1995 HGT Vol: 6 1531-1541) and the relative transduction efficiency compared. As shown in Figure 7, AAV5 transduced these cells 50- fold more efficiently than AAV2. This is the first time apical cells or cells exposed to the air have been shown to be infected by a gene therapy agent.

Transduction of striated muscle

Chicken myoblasts were cultured and plated as previously described (Rhodes & Yamada 1995 NAR Vol 23 (12) 2305-13). Cells were allowed to fuse and then transduced with a similar number of particles of rAAV2 or rAAV5 containing a nuclear localized β-gal transgene as previously described above after 5 days in culture. The cells were stained for β-gal activity following the procedure of (Chiorini et al. 1995 HGT Vol: 6 1531-1541) and the relative transduction efficiency compared. As shown in Figure 8, AAV5 transduced these cells approximately 16 fold more efficiently than AAV2.

Transduction of rat brain explants

Primary neonatal rat brain explants were prepared as previously described (Scortegagna et al. Neurotoxicology. 1997; 18 (2): 331-9). After 7 days in culture, cells were transduced with a similar number of particles of rAAV5 containing a nuclear localized β-gal transgene as previously described. After 5 days in culture, the cells were stained for β-gal activity following the procedure of (Chiorini et al. 1995 HGT Vol: 6 1531-1541). As shown in Figure 9, transduction was detected in a variety of cell types including astrocytes, neuronal cels and glial cells.

Transduction of human umbilical vein endothelial cells

Human umbilical vein endothelial cells were cultured and plated as previously described (Gnantenko et al. J Investig Med. 1997 Feb; 45(2): 87-98). Cells were transduced with rAAV2 or rAAV5 containing a nuclear localized β-gal transgene with 10 particles of virus/ cell (MOI 5) in minimal media then returned to complete media. After 24 hrs in culture the cells were stained for β-gal activity following the procedure of Chiorini et al. (1995 HGT Vol: 6 1531-1541), and the relative transduction efficiency compared. As shown in Figure 10, AAV5 transduced these cell 5-10 fold more efficiently than AAV2.

Throughout this application, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

Although the present process has been described with reference to specific details of certain embodiments thereof, it is not intended that such details should be regarded as limitations upon the scope of the invention except as and to the extent that they are included in the accompanying claims.

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What is claimed is:

1. A nucleic acid vector comprising a pair of adeno-associated virus 5 (AAV5) inverted terminal repeats and a promoter between the inverted terminal repeats.

2. The vector of claim 1, wherein the promoter is an AAV promoter p5.

3. The vector of claim 1, wherein the p5 promoter is AAV5 p5 promoter.

4. The vector of claim 1, further comprising an exogenous nucleic acid functionally linked to the promoter.

5. The vector of claim 1 encapsidated in an adeno-associated virus particle.

6. The particle of claim 5, wherein the particle is an AAV5 particle.

7. The particle of claim 5, wherein the particle is an AAV1 particle, an AAV2 particle, an AAV3 particle, an AAV4 particle or an AAV6 particle.

8. A recombinant AAV5 virion containing a vector comprising a pair of AAV5 inverted terminal repeats.

9. The virion of claim 8, wherein the vector further comprises an exogenous nucleic acid inserted between the inverted terminal repeats.

10. An isolated nucleic acid comprising the nucleotide sequence set forth in SEQ ID NO:1.

11. An isolated nucleic acid consisting essentially of the nucleotide sequence set forth in SEQ ID NO:1.

12. An isolated nucleic acid that selectively hybridizes with the nucleic acid of claim 11.

13. An isolated nucleic acid encoding an adeno-associated virus 5 Rep protein.

14. The nucleic acid of claim 13, wherein the adeno-associated virus 5 Rep protein has the amino acid sequence set forth in SEQ ID NO:2.

15. The nucleic acid of claim 13, wherein the adeno-associated virus 5 Rep protein has the amino acid sequence set forth in SEQ ID NO:3.

16. The nucleic acid of claim 13, wherein the adeno-associated virus 5 Rep protein has the amino acid sequence set forth in SEQ ID NO:12.

17. The nucleic acid of claim 13, wherein the adeno-associated virus 5 Rep protein has the amino acid sequence set forth in SEQ ID NO:14.

18. An isolated AAV 5 Rep protein.

19. The isolated AAV5 Rep protein of claim 18, having the amino acid sequence set forth in SEQ ID NO:2, or a unique fragment thereof.

20. The isolated AAV5 Rep protein of claim 18, having the amino acid sequence set forth in SEQ ID NO:3, or a unique fragment thereof.

21. An isolated antibody that specifically binds the protein of claim 18.

22. An isolated AAV5 capsid protein.

23. The isolated AAV5 capsid protein of claim 22 having the amino acid sequence set forth in SEQ ID NO:4.

24. An isolated antibody that specifically binds the protein of claim 23.

25. The isolated AAV5 capsid protein of claim 22, having the amino acid sequence set forth in SEQ ID NO:5.

26. An isolated antibody that specifically binds the protein of claim 25.

27. The isolated AAV5 capsid protein of claim 22, having the amino acid sequence set forth in SEQ ID NO:6.

28. An isolated antibody that specifically binds the protein of claim 27.

29. An isolated nucleic acid encoding the protein of claim 22.

30. The nucleic acid of claim 29, having the nucleic acid sequence set forth in SEQ ID NO:7.

31. The nucleic acid of claim 29, having the nucleic acid sequence set forth in SEQ ID NO:8.

32. The nucleic acid of claim 29, having the nucleic acid sequence set forth in SEQ ID NO:9.

33. An isolated nucleic acid that selectively hybridizes with the nucleic acid of claim 29.

34. An AAV5 particle comprising a capsid protein consisting essentially of the amino acid sequence set forth in SEQ ID NO:6.

35. An isolated nucleic acid comprising an AAV5 p5 promoter.

36. A method of screening a cell for infectivity by AAV5, comprising contacting the cell with AAV5 and detecting the presence of AAV5 in the cells.

37. A method of determining the suitability of an AAV5 vector for administration to a subject, comprising contacting an antibody-containing sample from the subject with an antigenic fragment of a protein of claim 22 and detecting an antibody-antigen reaction in the sample, the presence of a neutralizing reaction indicating the AAV5 vector to be unsuitable for use in the subject.

38. A method of determining the presence in a subject of an AAV5-specific antibody comprising, contacting an antibody-containing sample from the subject with an antigenic fragment of the protein of claim 22 and detecting an antibody-antigen reaction in the sample, the presence of a reaction indicating the presence of an AAV5-specific antibody in the subject.

39. A method of delivering a nucleic acid to a cell, comprising administering to the cell an AAV5 particle containing a vector comprising the nucleic acid inserted between a pair of AAV inverted terminal repeats, thereby delivering the nucleic acid to the cell.

40. The method of claim 39, wherein the AAV inverted terminal repeats are AAV5 inverted terminal repeats.

41. A method of delivering a nucleic acid to a subject comprising administering to a cell from the subject an AAV5 particle comprising the nucleic acid inserted between a

pair of AAV inverted terminal repeats, and returning the cell to the subject, thereby delivering the nucleic acid to the subject.

42. A method of delivering a nucleic acid to a cell in a subject comprising administering to the subject an AAV5 particle comprising the nucleic acid inserted between a pair of AAV inverted terminal repeats, thereby delivering the nucleic acid to a cell in the subject.

43. A method of delivering a nucleic acid to a cell in a subject having antibodies to AAV2 comprising administering to the subject an AAV5 particle comprising the nucleic acid, thereby delivering the nucleic acid to a cell in the subject.

44. An isolated nucleic acid comprising the nucleotide sequence set forth in SEQ ID NO:21.

45. An isolated nucleic acid comprising the nucleotide sequence set forth in SEQ ID NO: 23.

ABSTRACT OF THE DISCLOSURE

The present invention provides an adeno-associated virus 5 (AAV5) virus and vectors and particles derived therefrom. In addition, the present invention provides methods of delivering a nucleic acid to a cell using the AAV5 vectors and particles.

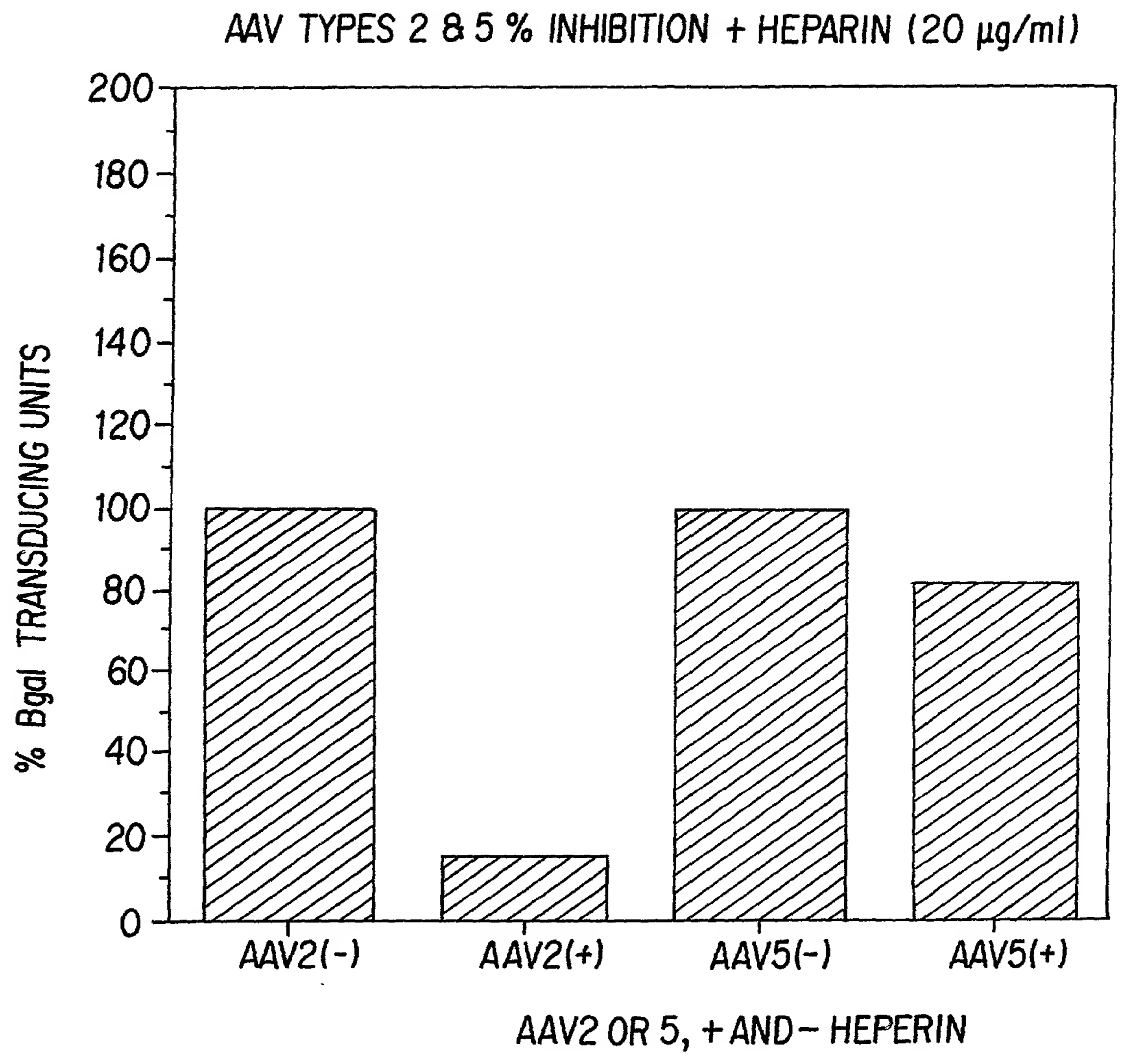

AAV TYPES 2 & 5 % INHIBITION + HEPARIN (20 µg/ml)
% Bgal TRANSDUCING UNITS
200
180
160
140
120
100
80
60
40
20
0
AAV2(-)
AAV2(+)
AAV5(-)
AAV5(+)
AAV2 OR 5, + AND - HEPERIN

FIG. 1

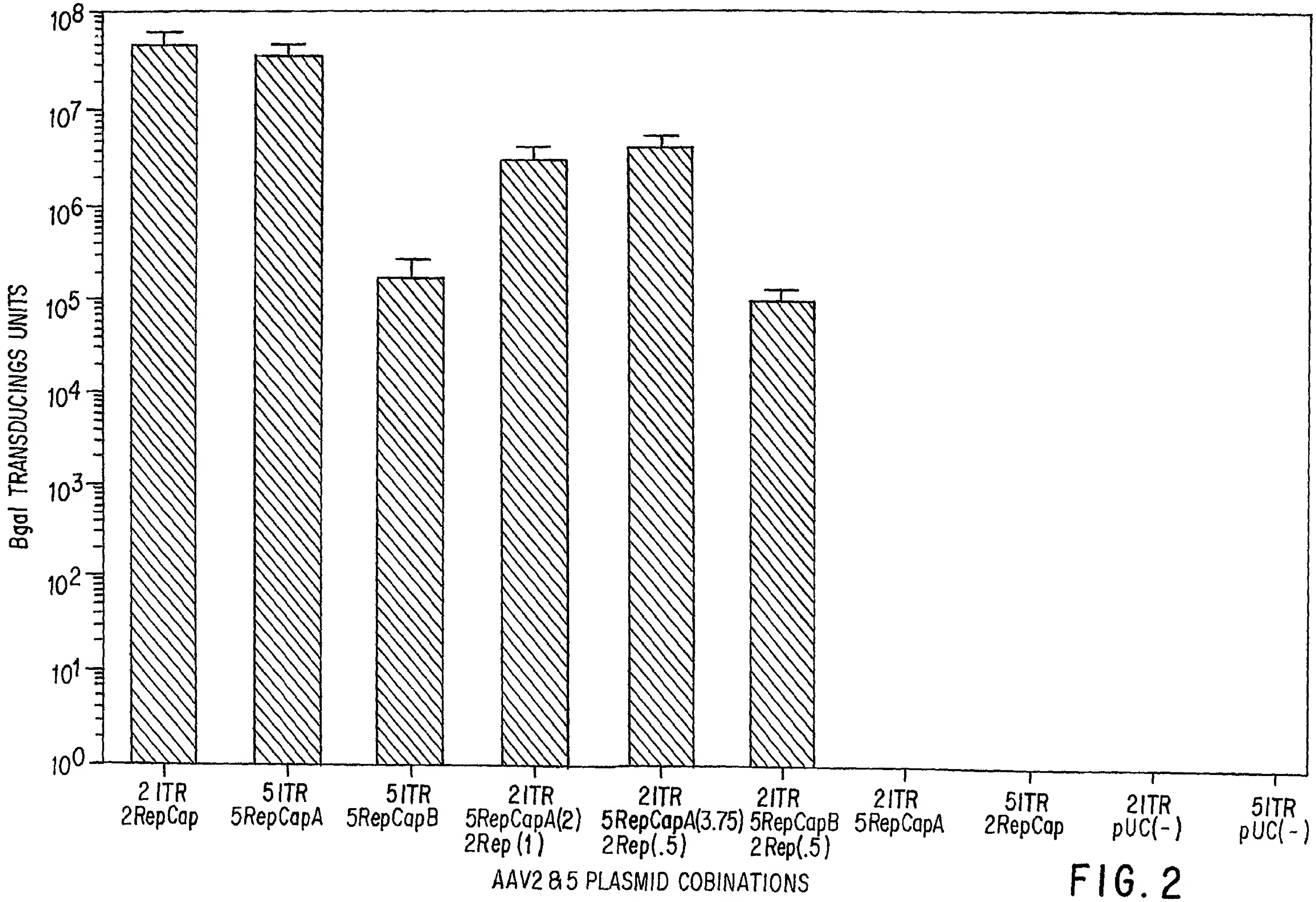
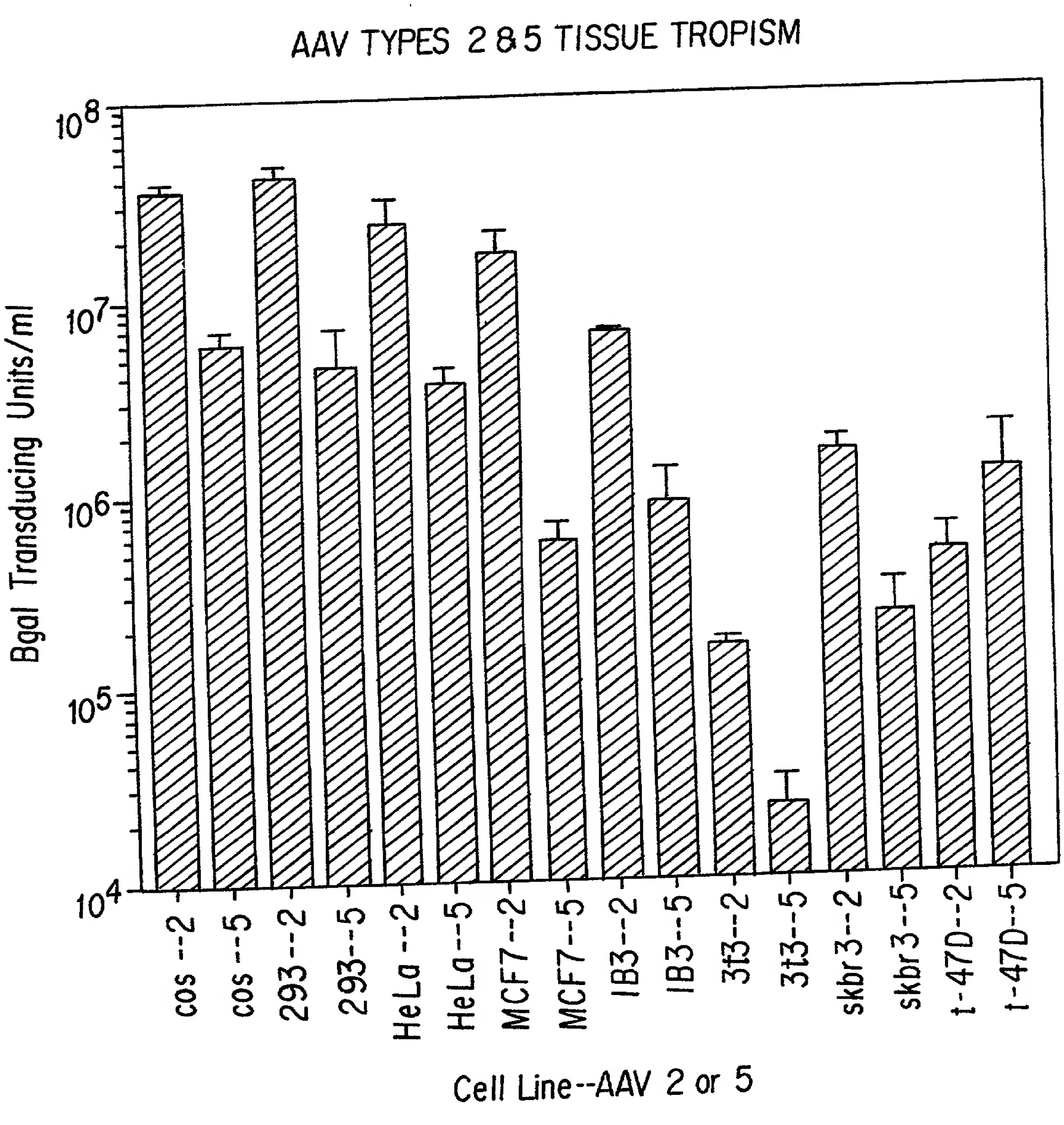

Bgal TRANSDUCINGS UNITS
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
2 ITR
2RepCap
5 ITR
5RepCapA
5 ITR
5RepCapB
2 ITR
5RepCapA(2)
2Rep (1)
2 ITR
5RepCapA(3.75)
2Rep(.5)
2 ITR
5RepCapB
2Rep(.5)
2 ITR
5RepCapA
5 ITR
2RepCap
2 ITR
pUC(-)
5 ITR
pUC(-)
AAV2 & 5 PLASMID COBINATIONS

FIG. 2



AAV TYPES 2 & 5 TISSUE TROPISM
Bgal Transducing Units/ml
10^8
10^7
10^6
10^5
10^4
cos --2
cos --5
293--2
293--5
HeLa --2
HeLa --5
MCF7--2
MCF7--5
IB3--2
IB3--5
3t3--2
3t3--5
skbr3--2
skbr3--5
t-47D--2
t-47D--5
Cell Line--AAV 2 or 5

FIG. 3

===23-SEP-1999================NALIGN====================PC/GENE===

```
******************************************
* ALIGNMENT OF TWO NUCLEOTIDE SEQUENCES. *
******************************************
```

The two sequences to be aligned are:

AAV2CG.
Total number of bases: 4679.

AAV5CG.
Total number of bases: 4652.

Open gap cost : 10
Unit gap cost : 12

The character to show that two aligned residues are identical is ':'

```
AAV2CG   - TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGA--------GGCCGGGCGA -48
           : :  : ::::: :    :::: :::::::::: :::         ::  ::: :
AAV5CG   - TGGCACTCTCCCCCCTGTCGCGTTCGCTCGCTCGCTGGCTCGTTTGGGGGGTGG -55

AAV2CG   - C----CAAAGGTC-GCCCGACGCCCGGGCTTTGCCCGG-GCGGCCTCA--------- -90
           :    :::::  : ::: :::: : :  :: :: :::  ::  :: ::
AAV5CG   - CAGCTCAAAGAGCTGCCAGACGACGGCCCTCTGGCCGTCGCCCCCCCAAACGAGC -110

AAV2CG   - --GTGAGCGAGCGAGCGCG-CAGAGAGG-GAGTGGCCAACTCCATCACTAGGGGT -141
            : :::::::::: :::: ::: : :: ::::: :  ::::     : :::::
AAV5CG   - CAGCGAGCGAGCGAACGCGACAGGGGGGAGAGTGCCACACTCTCAAGCAAGGGGG -165

AAV2CG   - TCCTGGAGGG-GTGGAGTCGTGACG-TGAATTACGTCATAGGGTTAGGGAGGTCC -194
           :  :: : :  :::  ::: : : : :: : : : :  :         :  :: :
AAV5CG   - TTTTGTAAGCAGTGATGTCATAATGATGTAATGCTTATTGTCACGCGATAGTTAA -220

AAV2CG   - TGTATTAGAGGTCACGTGA-GTGTTTTGCGACATTTTGCGACACC-------ATGT -242
           :: ::::   :::: :::: :::::::     ::      :: : :     :::
AAV5CG   - TG-ATTAACAGTCATGTGATGTGTTTTATCCAATAGGAAGAAAGCGCGCGTATGA -274

AAV2CG   - GGTCACGCT----------GGGTATTTAAGCCCGAGTGAGCACGCAGGGTCTCCAT -288
           : :: :::          :::::: ::: :::::::: :  ::  :  : ::::
AAV5CG   - GTTCTCGCGAGACTTCCGGGGTATAAAAGACCGAGTGAACGAGCCCGC-CGCCAT -328

AAV2CG   - T-TTGAAGCGGGAG-GTTTGAACGCGCA-GCCGCCATGCCGGGGTTTTACGAGAT -340
           : ::     : :::  : : ::   : :  :: ::::::: :     :: :: ::  :
AAV5CG   - TCTTTGCTCTGGACTGCTAGAGGACCCTCGCTGCCATGGCTACCTTCTATGAAGT -383
```

FIG.4A

```
AAV2CG    - TGTGATTAAGGTCCCCAGCGACCTTGACGGGCATCTGCCCGGCATTTCTGACAGC -395
              :  ::   :::::    ::: : :: :  :::::::: :: ::::::::::::
AAV5CG    - CATTGTTCGCGTCCCATTTGACGTGGAGGAACATCTGCCTGGAATTTCTGACAGC -438

AAV2CG    - TTTGTGAACTGCGTGGCCGAGAAGGAATGGGAGTTGCCGCCAGATTCTGACATGG -450
            :::::: :::::::  : :    :     :::::: :::: ::::: :: ::  :
AAV5CG    - TTTGTGGACTGGGTAACTGGTCAAATTTGGGAGCTGCCTCCAGAGTCAGATTTAA -493

AAV2CG    - ATCTGAATCTGATTGAGCAGGCACCCCTGACCGTGGCCGAGAAGCTGCAGCGCGA -505
            :: ::: :::: :::: ::: : :    :::: ::::: :: :    : :  ::::
AAV5CG    - ATTTGACTCTGGTTGAACAGCCTCAGTTGACGGTGGCTGATAGAATTCGCCGCGT -548

AAV2CG    - CTTTCTGACGGAATGGCGCCGTGTGAGTAAGGCCCCGGAGGCCCTTTTCTTTGTG -560
              :: :::   :: :::  :     :     :::   : :::: ::    :::::::::
AAV5CG    - GTTCCTGTACGAGTGGAACAAATTTTCCAAG---CAGGAGTCCAAATTCTTTGTG -600

AAV2CG    - CAATTTGAGAAGGGAGAGAGCTACTTCCACATGCACGTGCTCGTGGAAACCACCG -615
            :: ::::: ::::::       :: :: ::  :::::  ::: ::::: ::: :::
AAV5CG    - CAGTTTGAAAAGGGATCTGAATATTTTCATCTGCACACGCTTGTGGAGACCTCCG -655

AAV2CG    - GGGTGAAATCCATGGTTTTGGACGTTTCCTGAGTCAGATTCGCGAAAAACTGAT -670
            :  :    ::::::::  : :: :: : : :::::::::::::::    : ::: :
AAV5CG    - GCATCTCTTCCATGGTCCTCGGCCGCTACGTGAGTCAGATTCGCGCCCAGCTGGT -710

AAV2CG    - TCAGAGAATTTACCGCGGGATCGAGCCGACTTTGCCAAACTGGTTCGCGGTCACA -725
              :     : : ::  :: :: :: ::      :      ::::: ::::   ::::
AAV5CG    - GAAAGTGGTCTTCCAGGGAATTGAACCCCAGATCAACGACTGGGTCGCCATCACC -765

AAV2CG    - AAGACCAGAAATGGCGCCGGAGGCGGGAACAAGGTGGTGGATGAGTGCTACATCC -780
            :::   :  :: :::    :::: :   :: :::::::::::::      : :: :: :
AAV5CG    - AAGGTAAAGAAGGGC---GGAGCC---AATAAGGTGGTGGATTCTGGGTATATTC -814

AAV2CG    - CCAATTACTTGCTCCCCAAAACCCAGCCTGAGCTCCAGTGGGCGTGGACTAATAT -835
            ::   ::: :::: :: ::    ::: :: :::::  :::::::::::::: ::  :
AAV5CG    - CCGCCTACCTGCTGCCGAAGGTCCAACCGGAGCTTCAGTGGGCGTGGACAAACCT -869

AAV2CG    - GGAACAGTATTTAAGCGCCTGTTTGAATCTCACGGAGCGTAAACGGTTGGTGGCG -890
            :::  :::::  :   :::     :::::::   :::::: :::::: : :: :::
AAV5CG    - GGACGAGTATAAATTGGCCGCCCTGAATCTGGAGGAGCGCAAACGGCTCGTCGCG -924

AAV2CG    - CAGCATCTGACGCACGTGTCGCAGACGCAGGAGCAGAACAAAGAGAATCAGAATC -945
            :::  :::: :  :     :::::: :::  :  ::: :    :     :::  :
AAV5CG    - CAGTTTCTGGCAGAATCCTCGCAG-CGCTCG--CAGGAGGCGGCTTCGCAGCGTG -976
```

FIG.4B

```
AAV2CG   - CCAATTCTGATGCGCCGGTGATCAGATCAAAAACTTCAGCCAGGTACATGGAGCT -1000
AAV5CG   - AGTTCTCGGCTGACCCGGTCATCAAAAGCAAGACTTCCCAGAAATACATGGCGCT -1031

AAV2CG   - GGTCGGGTGGCTCGTGGACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAGGAG -1055
AAV5CG   - CGTCAACTGGCTCGTGGAGCACGGCATCACTTCCGAGAAGCAGTGGATCCAGGAA -1086

AAV2CG   - GACCAGGCCTCATACATCTCCTTCAATGCGGCCTCCAACTCGCGGTCCCAAATCA -1110
AAV5CG   - AATCAGGAGAGCTACCTCTCCTTCAACTCCACCGGCAACTCTCGGAGCCAGATCA -1141

AAV2CG   - AGGCTGCCTTGGACAATGCGGGAAAGATTATGAGCCTGACTAAAACCGCCCCCGA -1165
AAV5CG   - AGGCCGCGCTCGACAACGCGACCAAAATTATGAGTCTGACAAAAAGCGCGGTGGA -1196

AAV2CG   - CTACCTGGTGGGCCAGCAGCCCGTG-GAGGACATTTCCAGCAATCGGATTTATAA -1219
AAV5CG   - CTACCTCGTGGGG-AGCTCCGTTCCCGAGGACATTTCAAAAAACAGAATCTGGCA -1250

AAV2CG   - AATTTTGGAACTAAACGGGTACGATCCCCAATATGCGGCTTCCGTCTTTCTGGGA -1274
AAV5CG   - AATTTTTGAGATGAATGGCTACGACCCGGCCTACGCGGGATCCATCCTCTACGGC -1305

AAV2CG   - TGGGCCACGAAAAAGTTCGGCAAGAGGAACACCATCTGGCTGTTTGGGCCTGCAA -1329
AAV5CG   - TGGTGTCAGCGCTCCTTCAACAAGAGGAACACCGTCTGGCTCTACGGACCCGCCA -1360

AAV2CG   - CTACCGGGAAGACCAACATCGCGGAGGCCATAGCCCACACTGTGCCCTTCTACGG -1384
AAV5CG   - CGACCGGCAAGACCAACATCGCGGAGGCCATCGCCCACACTGTGCCCTTTTACGG -1415

AAV2CG   - GTGCGTAAACTGGACCAATGAGAACTTTCCCTTCAACGACTGTGTCGACAAGATG -1439
AAV5CG   - CTGCGTGAACTGGACCAATGAAAACTTTCCCTTTAATGACTGTGTGGACAAAATG -1470

AAV2CG   - GTGATCTGGTGGGAGGAGGGGAAGATGACCGCCAAGGTCGTGGAGTCGGCCAAAG -1494
AAV5CG   - CTCATTTGGTGGGAGGAGGGAAAGATGACCAACAAGGTGGTTGAATCCGCCAAGG -1525

AAV2CG   - CCATTCTCGGAGGAAGCAAGGTGCGCGTGGACCAGAAATGCAAGTCCTCGGCCCA -1549
AAV5CG   - CCATCCTGGGGGGCTCAAAGGTGCGGGTCGATCAGAAATGTAAATCCTCTGTTCA -1580
```

FIG.4C

```
AAV2CG  - GATAGACCCGACTCCCGTGATCGTCACCTCCAACACCAACATGTGCGCCGTGATT -1604
            :: ::  : :: :: :: :: :: :: ::::: :: :::::::: :  ::: :
AAV5CG  - AATTGATTCTACCCCTGTCATTGTAACTTCCAATACAAACATGTGTGTGGTGGTG -1635

AAV2CG  - GACGGGAACTCAACGACCTTCGAACACCAGCAGCCGTTGCAAGACCGGATGTTCA -1659
          :: ::::: :: :::::::: :::::::::::::: :: : ::::: :::::::
AAV5CG  - GATGGGAATTCCACGACCTTTGAACACCAGCAGCCGCTGGAGGACCGCATGTTCA -1690

AAV2CG  - AATTTGAACTCACCCGCCGTCTGGATCATGACTTTGGGAAGGTCACCAAGCAGGA -1714
          :::::::::: ::    :: ::    :  :: ::::: ::: : :: ::::::::
AAV5CG  - AATTTGAACTGACTAAGCGGCTCCCGCCAGATTTTGGCAAGATTACTAAGCAGGA -1745

AAV2CG  - AGTCAAAGACTTTTTCCGGTGGGCAAAGGATCACGTGGTTGAGGTGGAGCATGAA -1769
          :::::: ::::::::     ::::::::::  :   :::   ::::   :: ::
AAV5CG  - AGTCAAGGACTTTTTTGCTTGGGCAAAGGTCAATCAGGTGCCGGTGACTCACGAG -1800

AAV2CG  - TTCTACGTCAAAAAGGG—TGGAGCCAAGAAAAGACCCGCCCCCAGTGACGCAGA -1822
          :: : ::    : ::   ::: :  :: ::::   ::      : ::
AAV5CG  - TTTAAAGTTCCCAGGGAATTGGCGGGAACTAAAGGGGCG———GAGAAATCTC -1849

AAV2CG  - TATAAGTGAGCCCAAACGGGTGCGCGAGTCAGTTGCGCAGCCATCGACGTCAGAC -1877
          :: ::    :::::  :::::  ::   :   : :  ::: ::  : ::: :
AAV5CG. - TAAAAC——GCCCACT-GGGTGA-CGTCACCAATACT-AGCTATAAAAGTCTGGA -1898

AAV2CG  - GCGGAAGCTTCGATCAACTACGCAGACAGGTACCAAAACAAAT-GTTCTCGTCAC -1931
          :   ::::  :  ::   : ::   : : :: : ::   : :   :    ::
AAV5CG  - G——AAGC—GGGCCAGGCTCTCATTT-GTTCCCGAGACGCCTCGCAGTTCAGAC -1947

AAV2CG  - GTGGGCATGAATCT-GATGCTGTTTCCCTGCAGACAATGCGAGAGAATGAATCAG -1985
          :::    :  :::  : : :: :    : ::  : : ::  :   :: : ::  :
AAV5CG  - GTGACTGTTGATCCCGCTCCTCTGCGACCGCTCA-ATTGGAATTCAAGGTAT—G -1999

AAV2CG  - AATTCAAATATCTGCTTCACTCACGGACAGAAAGACTGTTTAGAGTGCTTTCCCG -2040
          : : :::::   ::  : : ::: :  ::    :::      : : : :: :
AAV5CG  - ATTGCAAATG—TGACT-A-TCATGCTCAATTTGACA———ACATTTCTAACAAA -2046

AAV2CG  - TGTCA-GAATCTCAACCCGTTTCTGTCGTCAAAAAGGC—GTATCAGAAACTGTG -2092
          ::: : :::: : ::    :    :    : :::::: :   ::::: :  ::  ::
AAV5CG  - TGTGATGAATGTGAATATTTGAATCGGGGCAAAAATGGATGTATCTGTCACAATG -2101

AAV2CG  - CTACATTCA-TCATAT———CATGGGAAAGGTGCCAGACGCTTGCACTGCCTGCG -2142
           ::   :  ::: ::     :::::::       :: :      : :    : ::
AAV5CG  - TAACTCACTGTCAAATTTGTCATGGGATTCCCCCCTGGGAAAAGGAAAACTTG— -2154

AAV2CG  - ATCTGGTCAATGTGGATTTGGATGACTGCATCTTTGAACAATAAATGATTTAAAT -2197
            :: : :   :: :::::: :: ::   ::     ::::: ::::: :      : :
AAV5CG  - -TCAGATTT-TGGGGATTTTGACGATGCCAATAAAGAACAGTAAATAAAGCGAGT -2207
```

FIG.4D

```
AAV2CG  - CAGGTATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACACTCTCTCTGA -2252
AAV5CG  - -AGTCATGTCTTTTGTTGATCACCCTCCAGATTGGTTGGAAGAAGTTGG---TGA -2258

AAV2CG  - AGGAATAAGACAGTGGTGGAAGCTCAAACCTGGCCCACCACCACCAAAGCCCGCA -2307
AAV5CG  - AGGTCTTCGCGAGTTTTTGGGCCTTGAAGCGGGCCCACCGAAACCAAAACCCAAT -2313

AAV2CG  - GAGCGGCATAAGGACGACAGCAGGGGTCTTGTGCTTCCTGGGTACAAGTACCTCG -2362
AAV5CG  - CAGCAGCATCAAGATCAAGCCCGTGGTCTTGTGCTGCCTGGTTATAACTATCTCG -2368

AAV2CG  - GACCCTTCAACGGACTCGACAAGGGAGAGCCGGTCAACGAGGCAGACGCCGCGGC -2417
AAV5CG  - GACCCGGAAACGGTCTCGATCGAGGAGAGCCTGTCAACAGGGCAGACGAGGTCGC -2423

AAV2CG  - CCTCGAGCACGACAAAGCCTACGACCGGCAGCTCGACAGCGGAGACAACCCGTAC -2472
AAV5CG  - GCGAGAGCACGACATCTCGTACAACGAGCAGCTTGAGGCGGGAGACAACCCCTAC -2478

AAV2CG  - CTCAAGTACAACCACGCCGACGCGGAGTTTCAGGAGCGCCTTAAAGAAGATACGT -2527
AAV5CG  - CTCAAGTACAACCACGCGGACGCCGAGTTTCAGGAGAAGCTCGCCGACGACACAT -2533

AAV2CG  - CTTTTGGGGGCAACCTCGGACGAGCAGTCTTCCAGGCGAAAAAGAGGGTTCTTGA -2582
AAV5CG  - CCTTCGGGGGAAACCTCGGAAAGGCAGTCTTTCAGGCCAAGAAAAGGGTTCTCGA -2588

AAV2CG  - ACCTCTGGGCCTGGTTGAGGAACCTGTTAAGACGGCTCCGGGAAAAAAGAGGCCG -2637
AAV5CG  - ACCTTTTGGCCTGGTTGAAGAGGGTGCTAAGACGGCCCCTACCGGAAAGCGGATA -2643

AAV2CG  - GTAGAGCACTCTCCTGTGGAGCCAGACTCCTCCTCGGGAACCGGAAAGGCGGGCC -2692
AAV5CG  - GACGACCACTTTCCAAAA-AGAAAGAAGGCTC----GGA-CCGAAGAGGACT-CC -2691

AAV2CG  - AGCAGCCTGCAAGAAAAAGATTGAATTTTGGTCAGACTGGAGACGCAG-ACTCAG -2746
AAV5CG  - A--AGCCTTCCACC--------------TCGTCAGAC-GCCGAAGCTGGACCCAG -2729

AAV2CG  - TACCTGACCCCCAGCCTCTCGGACAGCCACCAGCAGCCCCCTCTGGTCTGGGAAC -2801
AAV5CG  - ---CGGATCCC-AGCAGCTGCAAATCCCAGCCCAACCAGCCTCAAGTTTGGGAGC -2780
```

FIG.4E

```
AAV2CG   - TAATACGATGGCTACAGGCAGTGGCGCACCAATGGCAGACAATAACGAGGGCGCC -2856

AAV5CG   - TGATACAATGTCTGCGGGAGGTGGCGGCCCATTGGGCGACAATAACCAAGGTGCC -2835

AAV2CG   - GACGGAGTGGGTAATTCCTCGGGAAATTGGCATTGCGATTCCACATGGATGGGCG -2911

AAV5CG   - GATGGAGTGGGCAATGCCTCGGGAGATTGGCATTGCGATTCCACGTGGATGGGGG -2890

AAV2CG   - ACAGAGTCATCACCACCAGCACCCGAACCTGGGCCCTGCCCACCTACAACAACCA -2966

AAV5CG   - ACAGAGTCGTCACCAAGTCCACCCGAACCTGGGTGCTGCCCAGCTACAACAACCA -2945

AAV2CG   - CCTCTACAAACAAATTTCCAGCCAATCAGGAGCCTCGA---ACGACAATCACTAC -3018

AAV5CG   - CCAGTACCGAGAGATCAAAAGCGGCTCCGTCGACGGAAGCAACGCCAACGCCTAC -3000

AAV2CG   - TTTGGCTACAGCACCCCTTGGGGGTATTTTGACTTCAACAGATTCCACTGCCACT -3073

AAV5CG   - TTTGGATACAGCACCCCCTGGGGGTACTTTGACTTTAACCGCTTCCACAGCCACT -3055

AAV2CG   - TTTCACCACGTGACTGGCAAAGACTCATCAACAACAACTGGGGATTCCGACCCAA -3128

AAV5CG   - GGAGCCCCCGAGACTGGCAAAGACTCATCAACAACTACTGGGGCTTCAGACCCCG -3110

AAV2CG   - GAGACTCAACTTCAAGCTCTTTAACATTCAAGTCAAAGAGGTCACGCAGAATGAC -3183

AAV5CG   - GTCCCTCAGAGTCAAAATCTTCAACATTCAAGTCAAAGAGGTCACGGTGCAGGAC -3165

AAV2CG   - GGTACGACGACGATTGCCAATAACCTTACCAGCACGGTTCAGGTGTTTACTGACT -3238

AAV5CG   - TCCACCACCACCATCGCCAACAACCTCACCTCCACCGTCCAAGTGTTTACGGACG -3220

AAV2CG   - CGGAGTACCAGCTCCCGTACGTCCTCGGCTCGGCGCATCAAGGATGCCTCCCGCC -3293

AAV5CG   - ACGACTACCAGCTGCCCTACGTCGTCGGCAACGGGACCGAGGGATGCCTGCCGGC -3275

AAV2CG   - GTTCCCAGCAGACGTCTTCATGGTGCCACAGTATGGATACCTCACCCTGAACAAC -3348

AAV5CG   - CTTCCCTCCGCAGGTCTTTACGCTGCCGCAGTACGGTTACGCGACGCTGAACCGC -3330

AAV2CG   - GGGAGT-CAGGCAGTAGGAC---GCTCTTCA--TTTTACTGCCTGGAGTACTTTC -3397

AAV5CG   - GACAACACAGAAAATCCCACCGAGAGGAGCAGCTTCTTCTGCCTAGAGTACTTTC -3385
```

FIG.4F

```
AAV2CG   - CTTCTCAGATGCTGCGTACCGGAAACAACTTTACCTTCAGCTACACTTTTGAGGA -3452
           :     :::::::: : :: :: :::::::::    :: : :::::  ::::::::
AAV5CG   - CCAGCAAGATGCTGAGAACGGGCAACAACTTTGAGTTTACCTACAACTTTGAGGA -3440

AAV2CG   - CGTTCCTTTCCACAGCAGCTACGCTCACAGCCAGAGTCTGGACCGTCTCATGAAT -3507
            :: :: ::::::  ::::: ::::: ::: ::::  :::  :   ::    ::
AAV5CG   - GGTGCCCTTCCACTCCAGCTTCGCTCCCAGTCAGAACCTGTTCAAGCTGGCCAAC -3495

AAV2CG   - CCTCTCATCGACCAGTACCTGTATTACTT---GAGCAGAACAAACACTC------- -3553
           :: ::  : ::::::::: ::::    :::   ::::: ::  ::::::
AAV5CG   - CCGCTGGTGGACCAGTACTTGTACCGCTTCGTGAGCACAAATAACACTGGCGGAG -3550

AAV2CG   - -CAAGTGGAACCACCAC---GCAGTCA-AGGCTTCAGTT--TTCTCAGGCCGGAG -3601
            : :::  ::: :  ::   :: :  : :  :  ::     :  :  :  : ::
AAV5CG   - TCCAGTTCAACAAGAACCTGGCCGGGAGATACGCCAACACCTACAAAAACTGGTT -3605

AAV2CG   - CGAGTGACATTCGGGACCAGTCTAGGAACTGGCTTCCTGGACCCTGTTACCGCCA -3656
           :  : : :     ::: : :  : :::  ::::   ::::: : : :     :  :
AAV5CG   - CCCGGGGCCCATGGGCCGAACCCAGGG-CTGGAA-CCTGGGCTCCGGGGTCAACC -3658

AAV2CG   - GCAGCGAGTATCAAAGACATCTGCGGATAACAACAACAGTGAATACTCGTGGACT -3711
           :: :: ::: :::  : : :: ::     : ::: :  : :: :  :::: :: :
AAV5CG   - GC-GCCAGTGTCAGCGCCTTC-GCCACGACCAATAGGA-TGGAG-CTCGAGGGCG -3709

AAV2CG   - GGAGCTACCAAGTACCACCTCAATGGCAGAGACTCTCTGGTGAATCCGGGCCCGG -3766
            ::: ::::: :: :: :: ::   :: :: ::    ::   ::      :: ::
AAV5CG   - CGAGTTACCAGGTGCCCCCGCA---GCCGA-ACGGCATGACCAACAACCTCCAGG -3760

AAV2CG   - CCATGGCAAGCCACAAGGACGATGAAGAAAAGTTTTTTCCTCAGAGCGGGGTTCT -3821
           ::  ::::  :::      :  :: :::: : : :  :: :::   : :     :
AAV5CG   - GCA--GCAA--CACCTATGCCCTGGAGAACACTATGATCTTCAA--CAG----C- -3804

AAV2CG   - CATCTTTGGGAAGCAAGGCTCAGAGAAAACAAATGTGGACATTGAAAAGGTCATG -3876
           :: :   : ::: :  ::: :         ::  :  : ::     : : : ::::
AAV5CG   - CAGCCG-GCGAACCCGGGCACCACCGCCACGTACCTCGAGGGCAACATGCTCATC -3858

AAV2CG   - ATTACAGACGAAGAGGAAATCAGGACAACCAATCCCGTGGC-TACGGAGCAGTAT -3930
           :   ::: :::     :: :     : :    :: : :::::: :::   :  :
AAV5CG   - AC--CAG-CGAGAGCGAGACGCAGCCGGTGAACCGCGTGGCGTACAACGTCGGCG -3910

AAV2CG   - GGTTCTGTATCTACCAACCTCCAGAGAGGCAACAGACAAGCAGCTACCGCAGATG -3985
           ::      :  : :::::: ::::::  :: ::       : :: ::::
AAV5CG   - GGCAGA-TGGCCACCAACAACCAGAGCTCCACCACTGCCCCCGCGACCGGCACGT -3964
```

FIG.4G

```
AAV2CG  - TCAACACACAAGGCGTTCTTCCAGGCATGGTCTGGCAGGACAGAGATGTGTACCT -4040
AAV5CG  - ACAACCTCCAGGAAATCGTGCCCGGCAGCGTGTGGATGGAGAGGGACGTGTACCT -4019

AAV2CG  - TCAGGGGCCCATCTGGGCAAAGATTCCACACACGGACGGACATTTTCACCCCTCT -4095
AAV5CG  - CCAAGGACCCATCTGGGCCAAGATCCCAGAGACGGGGCGCACTTTCACCCCTCT -4074

AAV2CG  - CCCCTCATGGGTGGATTCGGACTTAAACACCCTCCTCCACAGATTCTCATCAAGA -4150
AAV5CG  - CCGGCCATGGGCGGATTCGGACTCAAACACCCACCGCCCATGATGCTCATCAAGA -4129

AAV2CG  - ACACCCCGGTACCTGCGAATCCTTCGACCACCTTCAGTG-CGGCAAAGTTTGCTT -4204
AAV5CG  - ACACGCCTGTGCCCGGAAATA--TC-ACCAGCTTCTCGGACGTGCCCGTCAGCAG -4181

AAV2CG  - CCTTCATCACACAGTACTCCACGGGACAGGTCAGCGTGGAGATCGAGTGGGAGCT -4259
AAV5CG  - C-TTCATCACCCAGTACAGCACCGGGCAGGTCACCGTGGAGATGGAGTGGGAGCT -4235

AAV2CG  - GCAGAAGGAAAACAGCAAACGCTGGAATCCCGAAATTCAGTACACTTCCAACTAC -4314
AAV5CG  - CAAGAAGGAAAACTCCAAGAGGTGGAACCCAGAGATCCAGTACACAAACAACTAC -4290

AAV2CG  - AACAAGTCTGTTAATGTGGACTTTACTGTGGACACTAATGGCGTGTATTCAGAGC -4369
AAV5CG  - AACGACCCCCAGTTTGTGGACTTTGCCCCGGACAGCACCGGGGA--ATACAGAAC -4343
```

FIG.4H

```
AAV2CG    - CTC--GCCCCATTGGCACCAGATACCTGACTCGTAATCTGTAAT---TGCTTGT- -4418
            : :  : :: :: :: ::: ::::::: :: ::     :: :::    : : :::
AAV5CG    - CACCAGACCTATCGGAACCCGATACCTTACCCGACCCCTTTAACCCATTCATGTC -4398

AAV2CG    - ---TAA--TCAATAAACCGTTTAATTCGTTTCAGTTGAACTTTGG-TCTCTGCGT -4467
               ::   ::::::::::: :: ::::: :::::  ::   ::  :::    ::
AAV5CG    - GCATACCCTCAATAAACCGTGTA-TTCGTGTCAGTAAAATACTGCCTCTTGTGGT -4452

AAV2CG    - ATTTCTTTCT-TATCTAGTTTCCATGGCTACGTAGATAAGTAGCATGGCGGGTTA -4521
              ::: :   :: :   :: : :   :::: :       : :: ::  : ::
AAV5CG    - CATTCAATGAATAACAGCTTACAACATCTACAAAACCTCCTTGCTTGA-GAGTGT -4506

AAV2CG    - ATCATTAACTACAAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTC-TCTGCGC -4575
              :: :  :: :      ::  : : : : :  : :  : ::  ::: : :: :
AAV5CG    - GGCACT--CTCCCC----CCTGTCGCGTTCGC-TCGCTCGCTGGCTCGTTTGGGG -4554

AAV2CG    - GCTCGCTCGCTCACTGAG--GCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTT -4628
            :   :   ::::: :::  ::: : ::::          : ::: :::::
AAV5CG    - GGGTGGCAGCTCAAAGAGCTGCCAGACGACGGCCCTCTGGCCGTCGCCCC----- -4604

AAV2CG    - TGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAA -4679
              :::   ::  : ::: :::::::::: ::::    : ::::: :  : :
AAV5CG    - --CCCAAACGAGC-CAGCGAGCGAGCGAACGCGACAGGGGGGAGAGTGCCA -4652
```

Identity : 3013 (64.77%)
Number of gaps inserted in AAV2CG: 43
Number of gaps inserted in AAV5CG: 63

===23-SEP-1999================NALIGN=====================PC/GENE===

FIG.4I

===23-SEP-1999==PC/GENE===

```
****************************************
* ALIGNMENT OF TWO PROTEIN SEQUENCES. *
****************************************
```

The two sequences to be aligned are:

AAV2VP1.
DE VP1
OS AAV2
Total number of residues: 735.

AAV5VP1.
DE AAV5VP1
OS AAV5VP1
Total number of residues: 724.

Comparison matrix : Structure-genetic matrix.
Open gap cost   : 8
Unit gap cost   : 5

The character to show that two aligned residues are identical is ':'
The character to show that two aligned residues are similar is '.'
Amino acids said to be 'similar' are: A,S,T; D,E; N,Q; R,K; I,L,M,V; F,Y,W

```
AAV2VP1  - MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDSRGLVLPGYKYLGP -55
           :.     :::::. . ::.: .  :  ::: :::   : : .:::::::: ::::
AAV5VP1  - MSFVDHPPDWLEE-VGEGLREFLGLEAGPPKPKPNQQHQDQARGLVLPGYNYLGP -54

AAV2VP1  - FNGLDKGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEFQERLKEDTSF -110
            ::::.::::: ::  : ::: .:  ::..:::::::::::::::::.: .::::
AAV5VP1  - GNGLDRGEPVNRADEVAREHDISYNEQLEAGDNPYLKYNHADAEFQEKLADDTSF -109

AAV2VP1  - GGNLGRAVFQAKKRVLEPLGLVEEPVKTAPGKKRPVEHSPVEPDSSSGTGKAGQQ -165
           :::::.:::::::::::: :::::  ::::  ::  .: :      . :
AAV5VP1  - GGNLGKAVFQAKKRVLEPFGLVEEGAKTAPTGKRIDDHFPKR--KKARTEEDSKP -162

AAV2VP1  - PARKRLNFGQTGDADSVPDPQPLGQPPAAPSGLGTNTMATGSGAPMADNNEGADG -220
            .        .         : :  :    : ::. ::..: : :. ::: ::::
AAV5VP1  - STS-------SDAEAGPSGSQQLQIPAQPASSLGADTMSAGGGGPLGDNNQGADG -210

AAV2VP1  - VGNSSGNWHCDSTWMGDRVITTSTRTWALPTYNNHLYKQISSQSG-ASNDNHYFG -274
           :::.:: ::::::::::::.: ::::: ::.:::: :. : : :   :: : :::
AAV5VP1  - VGNASGDWHCDSTWMGDRVVTKSTRTWVLPSYNNHQYREIKSGSVDGSNANAYFG -265
```

FIG.5A

```
AAV2VP1  - YSTPWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTQNDGT -329
           ::::::::::::: :.:::::::::: :::::. :  :.::::::::: .: :
AAV5VP1  - YSTPWGYFDFNRFHSHWSPRDWQRLINNYWGFRPRSLRVKIFNIQVKEVTVQDST -320

AAV2VP1  - TTIANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGS -384
           ::::::::::::::: .:::::.:    :::: ::  :: .::::: :::
AAV5VP1  - TTIANNLTSTVQVFTDDDYQLPYVVGNGTEGCLPAFPPQVFTLPQYGYATLNRDN -375

AAV2VP1  - Q--AVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPL -437
                :::::.::::::: :::::::: :.: ::.:::::::.: :: : .: :::
AAV5VP1  - TENPTERSSFFCLEYFPSKMLRTGNNFEFTYNFEEVPFHSSFAPSQNLFKLANPL -430

AAV2VP1  - IDQYLYYLSRTNTPSGTTTQSRLQFSQAGASDIRDQSRNWLPGPCYRQQRVSKTS -492
           .:::::    ::   :      .::     :        .:: :::  : :     :
AAV5VP1  - VDQYLYRFVSTNNTGG------VQFNKNLAGRYANTYKNWFPGPMGRTQGWNLGS -479

AAV2VP1  - ADNNNSEYSWTGATKYHLNGRDSLVNPGPAMASHKDDEEKFFPQSGVLIFGKQGS -547
             :  :  ... .  .  : :    : : :   .        .     .:: :  .
AAV5VP1  - GVNRASVSAFATTNRMELEGASYQVPPQPNGMTNNLQGSNTYALENTMIFNSQPA -534

AAV2VP1  - EKTNVDI---EKVMITDEEEIRTTNPVATEQYGSVSTNLQRGNRQAATADVNTQG -599
                           ..:: : :     : ::    : ..:: :        ::    : :
AAV5VP1  - NPGTTATYLEGNMLITSESETQPVNRVAYNVGGQMATNNQSSTTAPATGTYNLQE -589

AAV2VP1  - VLPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVP -654
           ..:: :: .::::::::::::: :  :::::: ::::::::::: .::::::::
AAV5VP1  - IVPGSVWMERDVYLQGPIWAKIPETGAHFHPSPAMGGFGLKHPPPMMLIKNTPVP -644

AAV2VP1  - ANPSTTFSAAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYNKSVN -709
             :  :.::    .::::::::::::.::.:::: ::::::::::::::: :::
AAV5VP1  - GNI-TSFSDVPVSSFITQYSTGQVTVEMEWELKKENSKRWNPEIQYTNNYNDPQF -698

AAV2VP1  - VDFTVDTNGVYSEPRPIGTRYLTRNL -735
           :::. :. : :  :::::::::: :
AAV5VP1  - VDFAPDSTGEYRTTRPIGTRYLTRPL -724
```

```
Identity  : 421 ( 58.2%)
Similarity: 63 ( 8.7%)
Number of gaps inserted in AAV2VP1: 3
Number of gaps inserted in AAV5VP1: 5
```

===23-SEP-1999==PC/GENE===

FIG.5B

```
===23-SEP-1999=================PALIGN====================PC/GENE===

****************************************
* ALIGNMENT OF TWO PROTEIN SEQUENCES. *
****************************************

The two sequences to be aligned are:

REP78.
DE   REP78
OS   AAV
Total number of residues: 621.

AAV5REP.
DE   REP
OS   AAV5
Total number of residues: 610.

Comparison matrix  : Structure-genetic matrix.
Open gap cost      : 8
Unit gap cost      : 5

The character to show that two aligned residues are identical is ':'
The character to show that two aligned residues are similar is '.'
Amino acids said to be 'similar' are: A,S,T; D,E; N,Q; R,K; I,L,M,V; F,Y,W


REP78    - MPGFYEIVIKVPSDLDGHLPGISDSFVNWVAEKEWELPPDSDMDLNLIEQAPLTV -55
           :  :::....:: :.. :::::::::: ::.   :::::.::. : :.::  :::
AAV5REP  - MATFYEVIVRVPFDVEEHLPGISDSFVDWVTGQIWELPPESDLNLTLVEQPQLTV -55

REP78    - AEKLQRDFLTEWRRVSKAPEALFFVQFEKGESYFHMHVLVETTGVKSMVLGRFLS -110
           :... : :: :: . ::  :. ::::::::  :::.: ::::.:. ::::::..:
AAV5REP  - ADRIRRVFLYEWNKFSKQ-ESKFFVQFEKGSEYFHLHTLVETSGISSMVLGRYVS -109

REP78    - QIREKLIQRIYRGIEPTLPNWFAVTKTRNGAGGGNKVVDECYIPNYLLPKTQPEL -165
           :::  :.  .. :::: .  : :.:: . :  : :::::  ::: ::::: ::::
AAV5REP  - QIRAQLVKVVFQGIEPQINDWVAITKVKKG--GANKVVDSGYIPAYLLPKVQPEL -162

REP78    - QWAWTNMEQYLSACLNLTERKRLVAQHLTHVSQTQEQNKENQNPNSDAPVIRSKT -220
           ::::::.. :  : ::: ::::::: :.  :    :    :   :  :::.:::
AAV5REP  - QWAWTNLDEYKLAALNLEERKRLVAQFLA-ESSQRSQEAASQREFSADPVIKSKT -216

REP78    - SARYMELVGWLVDKGITSEKQWIQEDQASYISFNAASNSRSQIKAALDNAGKIMS -275
           : .:: :: :::. :::::::::::: : ::.:::.. ::::::::::::: ::::
AAV5REP  - SQKYMALVNWLVEHGITSEKQWIQENQESYLSFNSTGNSRSQIKAALDNATKIMS -271
```

FIG.6A

```
REP78    - LTKTAPDYLVGQQPVEDISSNRIYKILELNGYDPQYAASVFLGWATKKFGKRNTI -330
           :::.: :::::    :::: :::. : :.:::: :: :.  ::  . : ::::.
AAV5REP  - LTKSAVDYLVGSSVPEDISKNRIWQIFEMNGYDPAYAGSILYGWCQRSFNKRNTV -326

REP78    - WLFGPATTGKTNIAEAIAHTVPFYGCVNWTNENFPFNDCVDKMVIWWEEGKMTAK -385
           ::.::::::::::::::::::::::::::::::::::::::::.:::::::::: :
AAV5REP  - WLYGPATTGKTNIAEAIAHTVPFYGCVNWTNENFPFNDCVDKMLIWWEEGKMTNK -381

REP78    - VVESAKAILGGSKVRVDQKCKSSAQIDPTPVIVTSNTNMCAVIDGNSTTFEHQQP -440
           :::::::::::::::::::::: ::: ::::::::::: :.:::::::::::::
AAV5REP  - VVESAKAILGGSKVRVDQKCKSSVQIDSTPVIVTSNTNMCVVVDGNSTTFEHQQP -436

REP78    - LQDRMFKFELTRRLDHDFGKVTKQEVKDFFRWAKDHVVEVEHEFYVKKGGAKKRP -495
           : ::::::::.::  ::::.::::::::: :::   : : ::: :     .
AAV5REP  - LEDRMFKFELTKRLPPDFGKITKQEVKDFFAWAKVNQVPVTHEFKV----PRELA -487

REP78    - APSDADISEPKRVRESVAQPSTSDAEASINYADRYQNKCSRHVGMNLMLFPCRQC -550
               :. :  ::    :.. :    :      .        : :  .    : :
AAV5REP  - GTKGAEKS-LKRPLGDVTNTSYKSLEKRARLSFVPETPRSSDVTVDPA--PLRPL -539

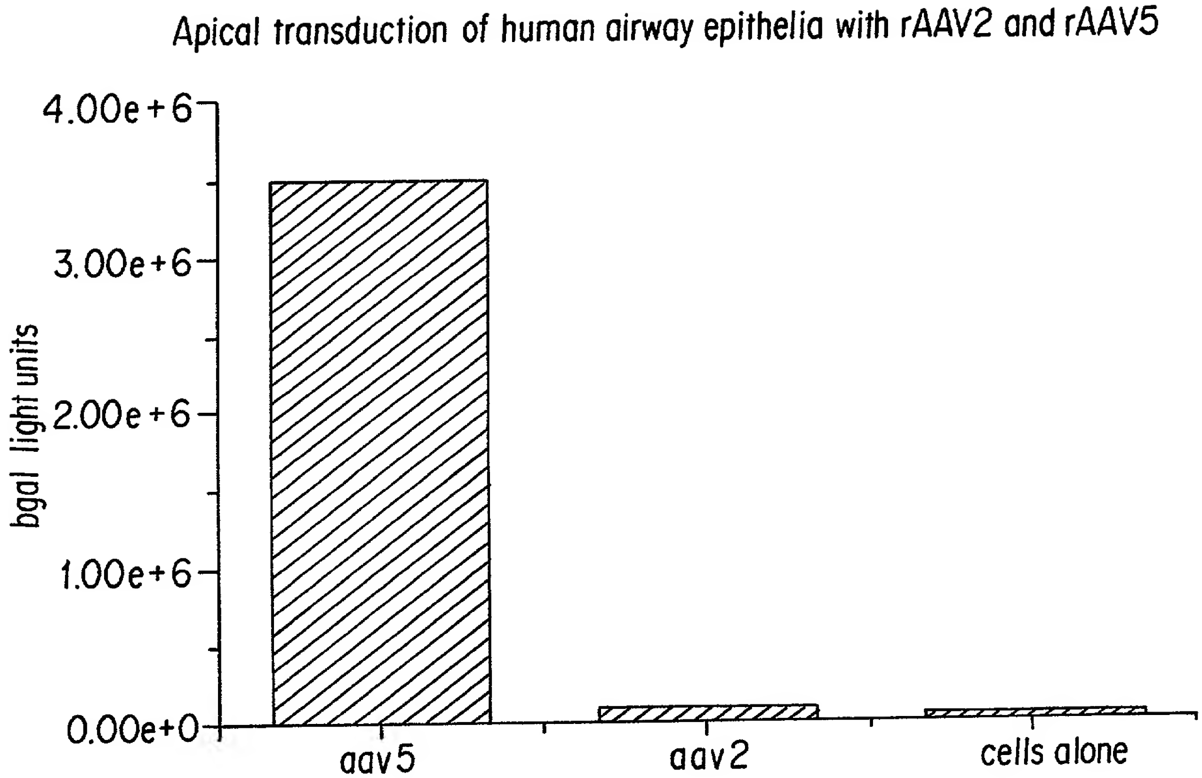
REP78    - ERMNQNSNICFTHGQKDCLECFPVSESQPVSVVKKAYQKLCYIHHIMGKVPDACT -605
                     :  : : : .      :    .    :         :  .
AAV5REP  - NWNSRYDCKCDYHAQFDNI-SNKCDECEYLNRGKNGCICHNVTHCQICHGIPPWE -593

REP78    - ACDLVNV-DLDDCIFEQ -621
               :    : ::   ::
AAV5REP  - KENLSDFGDFDDANKEQ -610
```

Identity : 363 (59.51%)
Similarity: 55 (9.02%)
Number of gaps inserted in REP78: 1
Number of gaps inserted in AAV5REP: 7

===23-SEP-1999================PALIGN======================PC/GENE===

FIG.6B


Apical transduction of human airway epithelia with rAAV2 and rAAV5
4.00e+6
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2.00e+6
1.00e+6
0.00e+0
bgal light units
aav5
aav2
cells alone

FIG. 7

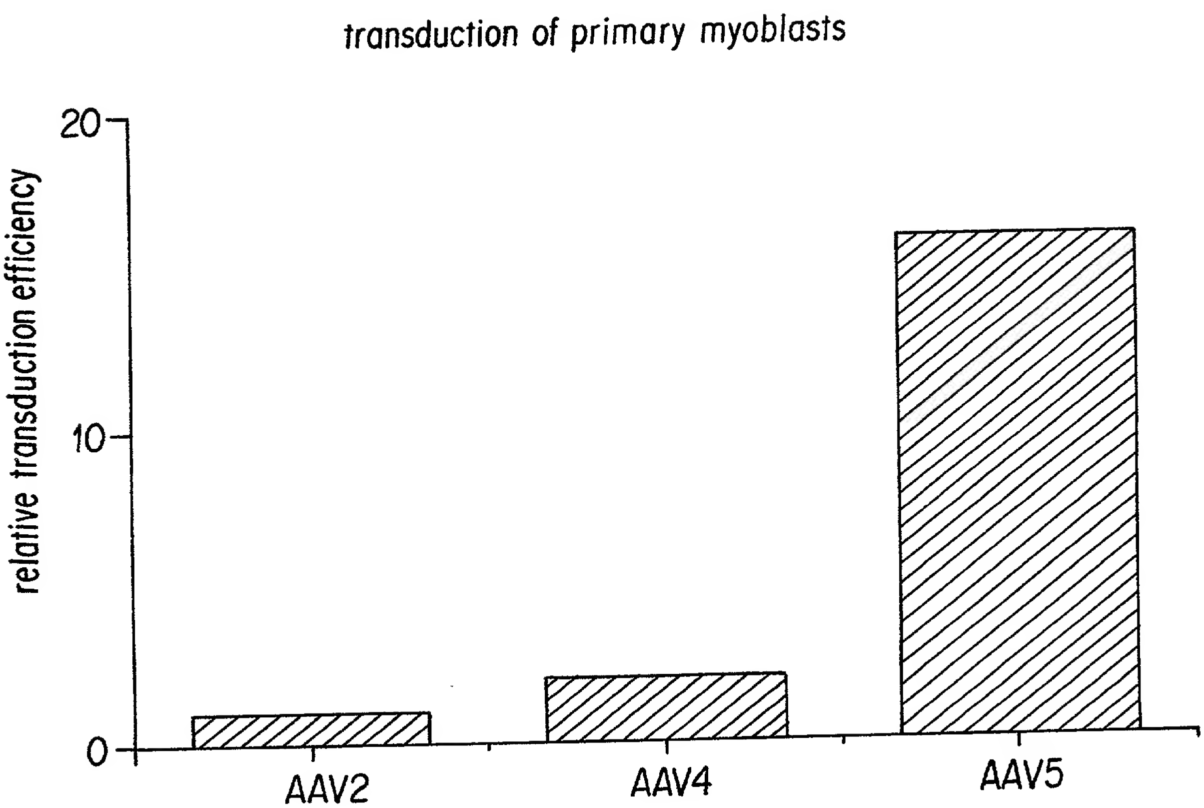
transduction of primary myoblasts
relative transduction efficiency
20
10
0
AAV2
AAV4
AAV5

FIG. 8

rAAV5 Primary Rat Brain Explant

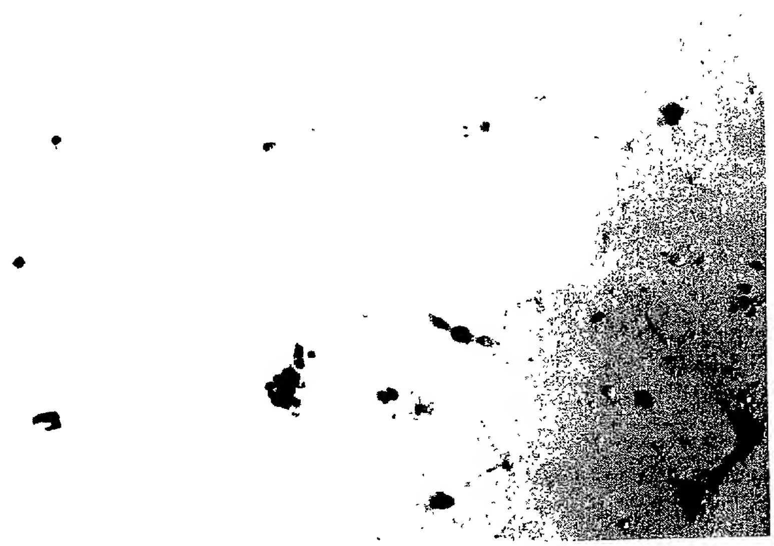

FIG.9

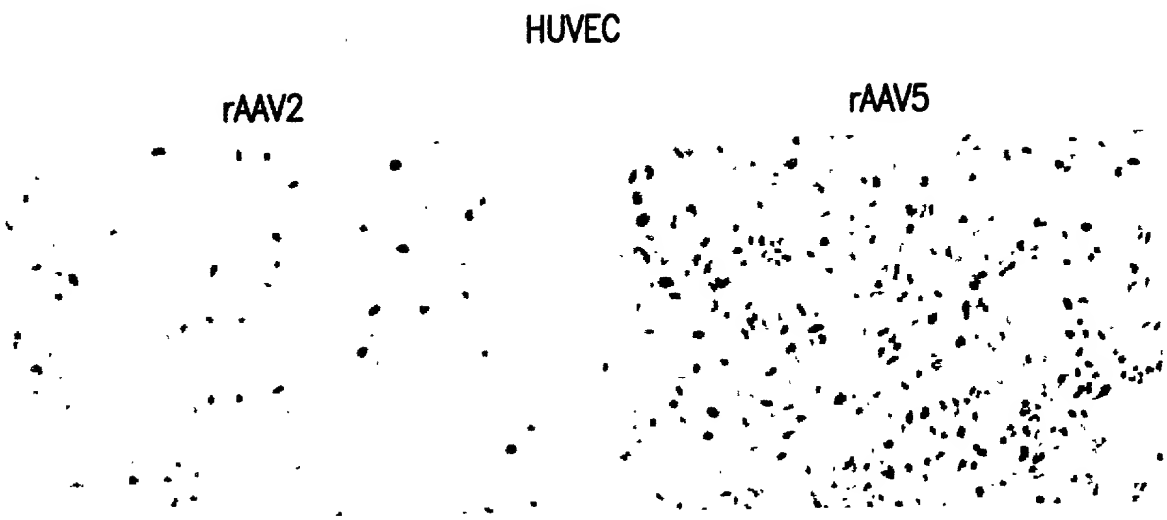

FIG.10

SEQUENCE LISTING

<110> Chiorini, John
Kotin, Robert M.
Safer, Brian

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synthetic construct

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gcgaacgcga cagggggag agtgccacac tctcaagcaa gggggttttg taagcagtga      180
tgtcataatg atgtaatgct tattgtcacg cgatagttaa tgattaacag tcatgtgatg    240
tgttttatcc aataggaaga aagcgcgcgt atgagttctc gcgagacttc cggggtataa    300
aagaccgagt gaacgagccc gccgccattc tttgctctgg actgctagag gaccctcgct    360
gccatggcta ccttctatga agtcattgtt cgcgtcccat ttgacgtgga ggaacatctg    420
cctggaattt ctgacagctt tgtggactgg gtaactggtc aaatttggga gctgcctcca    480
gagtcagatt taaatttgac tctggttgaa cagcctcagt tgacggtggc tgatagaatt    540
cgccgcgtgt tcctgtacga gtggaacaaa ttttccaagc aggagtccaa attctttgtg    600
cagtttgaaa aggatctgaa atattttcat ctgcacacgc ttgtggagac ctccggcatc    660
tcttccatgg tcctcggccg ctacgtgagt cagattcgcg cccagctggt gaaagtggtc    720
ttccagggaa ttgaacccca gatcaacgac tgggtcgcca tcaccaaggt aaagaagggc    780
ggagccaata aggtggtgga ttctgggtat attcccgcct acctgctgcc gaaggtccaa    840
ccggagcttc agtgggcgtg gacaaacctg gacgagtata aattggccgc cctgaatctg    900
gaggagcgca aacggctcgt cgcgcagttt ctggcagaat cctcgcagcg ctcgcaggag    960
gcggcttcgc agcgtgagtt ctcggctgac ccggtcatca aaagcaagac ttcccagaaa   1020
tacatggcgc tcgtcaactg gctcgtggag cacggcatca cttccgagaa gcagtggatc   1080
caggaaaatc aggagagcta cctctccttc aactccaccg gcaactctcg gagccagatc   1140
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gcggaggcca tcgcccacac tgtgcccttt tacggctgcg tgaactggac caatgaaaac 1440
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aacaaggtgg ttgaatccgc caaggccatc ctgggggggct caaaggtgcg ggtcgatcag 1560
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gttcccgaga cgcctcgcag ttcagacgtg actgttgatc ccgctcctct gcgaccgctc 1980
aattggaatt caaggtatga ttgcaaatgt gactatcatg ctcaatttga caacatttct 2040
aacaaatgtg atgaatgtga atatttgaat cggggcaaaa atggatgtat ctgtcacaat 2100
gtaactcact gtcaaatttg tcatgggatt ccccccctggg aaaaggaaaa cttgtcagat 2160
tttggggatt ttgacgatgc caataaagaa cagtaaataa agcgagtagt catgtctttt 2220
gttgatcacc ctccagattg gttggaagaa gttggtgaag gtcttcgcga gtttttgggc 2280
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gagaacacta tgatcttcaa cagccagccg gcgaacccgg gcaccaccgc cacgtacctc 3840
gagggcaaca tgctcatcac cagcgagagc gagacgcagc cggtgaaccg cgtggcgtac 3900
aacgtcggcg ggcagatggc caccaacaac cagagctcca ccactgcccc cgcgaccggc 3960
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atgggcggat tcggactcaa acacccaccg cccatgatgc tcatcaagaa cacgcctgtg 4140
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aacccagaga tccagtacac aaacaactac aacgaccccc agtttgtgga ctttgccccg 4320
gacagcaccg gggaatacag aaccaccaga cctatcggaa cccgatacct tacccgaccc 4380
ctttaaccca ttcatgtcgc ataccctcaa taaaccgtgt attcgtgtca gtaaaatact 4440
gcctcttgtg gtcattcaat gaataacagc ttacaacatc tacaaaacct ccttgcttga 4500
gagtgtggca ctctcccccc tgtcgcgttc gctcgctcgc tggctcgttt gggggggtgg 4560
cagctcaaag agctgccaga cgacggccct ctggccgtcg cccccccaaa cgagccagcg 4620
agcgagcgaa cgcgacaggg gggagagtgc ca 4652

<210> 2
<211> 390
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
synthetic construct

<400> 2
Met Ala Leu Val Asn Trp Leu Val Glu His Gly Ile Thr Ser Glu Lys
1 5 10 15
Gln Trp Ile Gln Glu Asn Gln Glu Ser Tyr Leu Ser Phe Asn Ser Thr
20 25 30
Gly Asn Ser Arg Ser Gln Ile Lys Ala Ala Leu Asp Asn Ala Thr Lys
35 40 45
Ile Met Ser Leu Thr Lys Ser Ala Val Asp Tyr Leu Val Gly Ser Ser
50 55 60
Val Pro Glu Asp Ile Ser Lys Asn Arg Ile Trp Gln Ile Phe Glu Met
65 70 75 80
Asn Gly Tyr Asp Pro Ala Tyr Ala Gly Ser Ile Leu Tyr Gly Trp Cys
85 90 95
Gln Arg Ser Phe Asn Lys Arg Asn Thr Val Trp Leu Tyr Gly Pro Ala
100 105 110
Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Thr Val Pro
115 120 125
Phe Tyr Gly Cys Val Asn Trp Thr Asn Glu Asn Phe Pro Phe Asn Asp
130 135 140
Cys Val Asp Lys Met Leu Ile Trp Trp Glu Glu Gly Lys Met Thr Asn
145 150 155 160
Lys Val Val Glu Ser Ala Lys Ala Ile Leu Gly Gly Ser Lys Val Arg
165 170 175
Val Asp Gln Lys Cys Lys Ser Ser Val Gln Ile Asp Ser Thr Pro Val
180 185 190
Ile Val Thr Ser Asn Thr Asn Met Cys Val Val Val Asp Gly Asn Ser
195 200 205
Thr Thr Phe Glu His Gln Gln Pro Leu Glu Asp Arg Met Phe Lys Phe
210 215 220
Glu Leu Thr Lys Arg Leu Pro Pro Asp Phe Gly Lys Ile Thr Lys Gln
225 230 235 240
Glu Val Lys Asp Phe Phe Ala Trp Ala Lys Val Asn Gln Val Pro Val
245 250 255

Thr His Glu Phe Lys Val Pro Arg Glu Leu Ala Gly Thr Lys Gly Ala
260 265 270
Glu Lys Ser Leu Lys Arg Pro Leu Gly Asp Val Thr Asn Thr Ser Tyr
275 280 285
Lys Ser Leu Glu Lys Arg Ala Arg Leu Ser Phe Val Pro Glu Thr Pro
290 295 300
Arg Ser Ser Asp Val Thr Val Asp Pro Ala Pro Leu Arg Pro Leu Asn
305 310 315 320
Trp Asn Ser Arg Tyr Asp Cys Lys Cys Asp Tyr His Ala Gln Phe Asp
325 330 335
Asn Ile Ser Asn Lys Cys Asp Glu Cys Glu Tyr Leu Asn Arg Gly Lys
340 345 350
Asn Gly Cys Ile Cys His Asn Val Thr His Cys Gln Ile Cys His Gly
355 360 365
Ile Pro Pro Trp Glu Lys Glu Asn Leu Ser Asp Phe Gly Asp Phe Asp
370 375 380
Asp Ala Asn Lys Glu Gln
385 390

<210> 3
<211> 610
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
synthetic construct

<400> 3
Met Ala Thr Phe Tyr Glu Val Ile Val Arg Val Pro Phe Asp Val Glu
1 5 10 15
Glu His Leu Pro Gly Ile Ser Asp Ser Phe Val Asp Trp Val Thr Gly
20 25 30
Gln Ile Trp Glu Leu Pro Pro Glu Ser Asp Leu Asn Leu Thr Leu Val
35 40 45
Glu Gln Pro Gln Leu Thr Val Ala Asp Arg Ile Arg Arg Val Phe Leu
50 55 60
Tyr Glu Trp Asn Lys Phe Ser Lys Gln Glu Ser Lys Phe Phe Val Gln
65 70 75 80
Phe Glu Lys Gly Ser Glu Tyr Phe His Leu His Thr Leu Val Glu Thr
85 90 95
Ser Gly Ile Ser Ser Met Val Leu Gly Arg Tyr Val Ser Gln Ile Arg
100 105 110
Ala Gln Leu Val Lys Val Val Phe Gln Gly Ile Glu Pro Gln Ile Asn
115 120 125
Asp Trp Val Ala Ile Thr Lys Val Lys Lys Gly Gly Ala Asn Lys Val
130 135 140
Val Asp Ser Gly Tyr Ile Pro Ala Tyr Leu Leu Pro Lys Val Gln Pro
145 150 155 160
Glu Leu Gln Trp Ala Trp Thr Asn Leu Asp Glu Tyr Lys Leu Ala Ala
165 170 175

Leu Asn Leu Glu Glu Arg Lys Arg Leu Val Ala Gln Phe Leu Ala Glu
180 185 190
Ser Ser Gln Arg Ser Gln Glu Ala Ala Ser Gln Arg Glu Phe Ser Ala
195 200 205
Asp Pro Val Ile Lys Ser Lys Thr Ser Gln Lys Tyr Met Ala Leu Val
210 215 220
Asn Trp Leu Val Glu His Gly Ile Thr Ser Glu Lys Gln Trp Ile Gln
225 230 235 240
Glu Asn Gln Glu Ser Tyr Leu Ser Phe Asn Ser Thr Gly Asn Ser Arg
245 250 255
Ser Gln Ile Lys Ala Ala Leu Asp Asn Ala Thr Lys Ile Met Ser Leu
260 265 270
Thr Lys Ser Ala Val Asp Tyr Leu Val Gly Ser Ser Val Pro Glu Asp
275 280 285
Ile Ser Lys Asn Arg Ile Trp Gln Ile Phe Glu Met Asn Gly Tyr Asp
290 295 300
Pro Ala Tyr Ala Gly Ser Ile Leu Tyr Gly Trp Cys Gln Arg Ser Phe
305 310 315 320
Asn Lys Arg Asn Thr Val Trp Leu Tyr Gly Pro Ala Thr Thr Gly Lys
325 330 335
Thr Asn Ile Ala Glu Ala Ile Ala His Thr Val Pro Phe Tyr Gly Cys
340 345 350
Val Asn Trp Thr Asn Glu Asn Phe Pro Phe Asn Asp Cys Val Asp Lys
355 360 365
Met Leu Ile Trp Trp Glu Glu Gly Lys Met Thr Asn Lys Val Val Glu
370 375 380
Ser Ala Lys Ala Ile Leu Gly Gly Ser Lys Val Arg Val Asp Gln Lys
385 390 395 400
Cys Lys Ser Ser Val Gln Ile Asp Ser Thr Pro Val Ile Val Thr Ser
405 410 415
Asn Thr Asn Met Cys Val Val Val Asp Gly Asn Ser Thr Thr Phe Glu
420 425 430

His Gln Gln Pro Leu Glu Asp Arg Met Phe Lys Phe Glu Leu Thr Lys
435 440 445
Arg Leu Pro Pro Asp Phe Gly Lys Ile Thr Lys Gln Glu Val Lys Asp
450 455 460
Phe Phe Ala Trp Ala Lys Val Asn Gln Val Pro Val Thr His Glu Phe
465 470 475 480
Lys Val Pro Arg Glu Leu Ala Gly Thr Lys Gly Ala Glu Lys Ser Leu
485 490 495
Lys Arg Pro Leu Gly Asp Val Thr Asn Thr Ser Tyr Lys Ser Leu Glu
500 505 510
Lys Arg Ala Arg Leu Ser Phe Val Pro Glu Thr Pro Arg Ser Ser Asp
515 520 525
Val Thr Val Asp Pro Ala Pro Leu Arg Pro Leu Asn Trp Asn Ser Arg
530 535 540
Tyr Asp Cys Lys Cys Asp Tyr His Ala Gln Phe Asp Asn Ile Ser Asn
545 550 555 560
Lys Cys Asp Glu Cys Glu Tyr Leu Asn Arg Gly Lys Asn Gly Cys Ile
565 570 575

Cys His Asn Val Thr His Cys Gln Ile Cys His Gly Ile Pro Pro Trp
580 585 590
Glu Lys Glu Asn Leu Ser Asp Phe Gly Asp Phe Asp Asp Ala Asn Lys
595 600 605
Glu Gln
610

<210> 4
<211> 724
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
synthetic construct

<400> 4
Met Ser Phe Val Asp His Pro Pro Asp Trp Leu Glu Glu Val Gly Glu
1 5 10 15
Gly Leu Arg Glu Phe Leu Gly Leu Glu Ala Gly Pro Pro Lys Pro Lys
20 25 30
Pro Asn Gln Gln His Gln Asp Gln Ala Arg Gly Leu Val Leu Pro Gly
35 40 45
Tyr Asn Tyr Leu Gly Pro Gly Asn Gly Leu Asp Arg Gly Glu Pro Val
50 55 60
Asn Arg Ala Asp Glu Val Ala Arg Glu His Asp Ile Ser Tyr Asn Glu
65 70 75 80
Gln Leu Glu Ala Gly Asp Asn Pro Tyr Leu Lys Tyr Asn His Ala Asp
85 90 95
Ala Glu Phe Gln Glu Lys Leu Ala Asp Asp Thr Ser Phe Gly Gly Asn
100 105 110
Leu Gly Lys Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro Phe
115 120 125
Gly Leu Val Glu Glu Gly Ala Lys Thr Ala Pro Thr Gly Lys Arg Ile
130 135 140
Asp Asp His Phe Pro Lys Arg Lys Lys Ala Arg Thr Glu Glu Asp Ser
145 150 155 160
Lys Pro Ser Thr Ser Ser Asp Ala Glu Ala Gly Pro Ser Gly Ser Gln
165 170 175

Gln Leu Gln Ile Pro Ala Gln Pro Ala Ser Ser Leu Gly Ala Asp Thr
180 185 190
Met Ser Ala Gly Gly Gly Gly Pro Leu Gly Asp Asn Asn Gln Gly Ala
195 200 205
Asp Gly Val Gly Asn Ala Ser Gly Asp Trp His Cys Asp Ser Thr Trp
210 215 220
Met Gly Asp Arg Val Val Thr Lys Ser Thr Arg Thr Trp Val Leu Pro
225 230 235 240
Ser Tyr Asn Asn His Gln Tyr Arg Glu Ile Lys Ser Gly Ser Val Asp
245 250 255
Gly Ser Asn Ala Asn Ala Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr

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            260                 265                 270
Phe Asp Phe Asn Arg Phe His Ser His Trp Ser Pro Arg Asp Trp Gln
        275                 280                 285
Arg Leu Ile Asn Asn Tyr Trp Gly Phe Arg Pro Arg Ser Leu Arg Val
    290                 295                 300
Lys Ile Phe Asn Ile Gln Val Lys Glu Val Thr Val Gln Asp Ser Thr
305                 310                 315                 320
Thr Thr Ile Ala Asn Asn Leu Thr Ser Thr Val Gln Val Phe Thr Asp
                325                 330                 335
Asp Asp Tyr Gln Leu Pro Tyr Val Val Gly Asn Gly Thr Glu Gly Cys
            340                 345                 350
Leu Pro Ala Phe Pro Pro Gln Val Phe Thr Leu Pro Gln Tyr Gly Tyr
        355                 360                 365
Ala Thr Leu Asn Arg Asp Asn Thr Glu Asn Pro Thr Glu Arg Ser Ser
    370                 375                 380
Phe Phe Cys Leu Glu Tyr Phe Pro Ser Lys Met Leu Arg Thr Gly Asn
385                 390                 395                 400
Asn Phe Glu Phe Thr Tyr Asn Phe Glu Glu Val Pro Phe His Ser Ser
                405                 410                 415
Phe Ala Pro Ser Gln Asn Leu Phe Lys Leu Ala Asn Pro Leu Val Asp
            420                 425                 430
Gln Tyr Leu Tyr Arg Phe Val Ser Thr Asn Asn Thr Gly Gly Val Gln
        435                 440                 445
Phe Asn Lys Asn Leu Ala Gly Arg Tyr Ala Asn Thr Tyr Lys Asn Trp
    450                 455                 460
Phe Pro Gly Pro Met Gly Arg Thr Gln Gly Trp Asn Leu Gly Ser Gly
465                 470                 475                 480
Val Asn Arg Ala Ser Val Ser Ala Phe Ala Thr Thr Asn Arg Met Glu
                485                 490                 495
Leu Glu Gly Ala Ser Tyr Gln Val Pro Pro Gln Pro Asn Gly Met Thr
            500                 505                 510
Asn Asn Leu Gln Gly Ser Asn Thr Tyr Ala Leu Glu Asn Thr Met Ile
        515                 520                 525
Phe Asn Ser Gln Pro Ala Asn Pro Gly Thr Thr Ala Thr Tyr Leu Glu
    530                 535                 540
Gly Asn Met Leu Ile Thr Ser Glu Ser Glu Thr Gln Pro Val Asn Arg
545                 550                 555                 560
Val Ala Tyr Asn Val Gly Gly Gln Met Ala Thr Asn Asn Gln Ser Ser
                565                 570                 575
Thr Thr Ala Pro Ala Thr Gly Thr Tyr Asn Leu Gln Glu Ile Val Pro
            580                 585                 590
Gly Ser Val Trp Met Glu Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp
        595                 600                 605
Ala Lys Ile Pro Glu Thr Gly Ala His Phe His Pro Ser Pro Ala Met
    610                 615                 620
Gly Gly Phe Gly Leu Lys His Pro Pro Pro Met Met Leu Ile Lys Asn
625                 630                 635                 640
Thr Pro Val Pro Gly Asn Ile Thr Ser Phe Ser Asp Val Pro Val Ser
                645                 650                 655
Ser Phe Ile Thr Gln Tyr Ser Thr Gly Gln Val Thr Val Glu Met Glu
            660                 665                 670
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Trp Glu Leu Lys Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Ile Gln
675 680 685
Tyr Thr Asn Asn Tyr Asn Asp Pro Gln Phe Val Asp Phe Ala Pro Asp
690 695 700
Ser Thr Gly Glu Tyr Arg Thr Thr Arg Pro Ile Gly Thr Arg Tyr Leu
705 710 715 720
Thr Arg Pro Leu

<210> 5
<211> 588
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
synthetic construct

<400> 5
Thr Ala Pro Thr Gly Lys Arg Ile Asp Asp His Phe Pro Lys Arg Lys
1 5 10 15
Lys Ala Arg Thr Glu Glu Asp Ser Lys Pro Ser Thr Ser Ser Asp Ala
20 25 30
Glu Ala Gly Pro Ser Gly Ser Gln Gln Leu Gln Ile Pro Ala Gln Pro
35 40 45
Ala Ser Ser Leu Gly Ala Asp Thr Met Ser Ala Gly Gly Gly Gly Pro
50 55 60
Leu Gly Asp Asn Asn Gln Gly Ala Asp Gly Val Gly Asn Ala Ser Gly
65 70 75 80
Asp Trp His Cys Asp Ser Thr Trp Met Gly Asp Arg Val Val Thr Lys
85 90 95
Ser Thr Arg Thr Trp Val Leu Pro Ser Tyr Asn Asn His Gln Tyr Arg
100 105 110
Glu Ile Lys Ser Gly Ser Val Asp Gly Ser Asn Ala Asn Ala Tyr Phe
115 120 125
Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg Phe His Ser
130 135 140
His Trp Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn Tyr Trp Gly
145 150 155 160
Phe Arg Pro Arg Ser Leu Arg Val Lys Ile Phe Asn Ile Gln Val Lys
165 170 175
Glu Val Thr Val Gln Asp Ser Thr Thr Thr Ile Ala Asn Asn Leu Thr
180 185 190
Ser Thr Val Gln Val Phe Thr Asp Asp Asp Tyr Gln Leu Pro Tyr Val
195 200 205
Val Gly Asn Gly Thr Glu Gly Cys Leu Pro Ala Phe Pro Pro Gln Val
210 215 220
Phe Thr Leu Pro Gln Tyr Gly Tyr Ala Thr Leu Asn Arg Asp Asn Thr
225 230 235 240
Glu Asn Pro Thr Glu Arg Ser Ser Phe Phe Cys Leu Glu Tyr Phe Pro
245 250 255

Ser Lys Met Leu Arg Thr Gly Asn Asn Phe Glu Phe Thr Tyr Asn Phe
260 265 270

Glu Glu Val Pro Phe His Ser Ser Phe Ala Pro Ser Gln Asn Leu Phe
275 280 285
Lys Leu Ala Asn Pro Leu Val Asp Gln Tyr Leu Tyr Arg Phe Val Ser
290 295 300
Thr Asn Asn Thr Gly Gly Val Gln Phe Asn Lys Asn Leu Ala Gly Arg
305 310 315 320
Tyr Ala Asn Thr Tyr Lys Asn Trp Phe Pro Gly Pro Met Gly Arg Thr
325 330 335
Gln Gly Trp Asn Leu Gly Ser Gly Val Asn Arg Ala Ser Val Ser Ala
340 345 350
Phe Ala Thr Thr Asn Arg Met Glu Leu Glu Gly Ala Ser Tyr Gln Val
355 360 365
Pro Pro Gln Pro Asn Gly Met Thr Asn Asn Leu Gln Gly Ser Asn Thr
370 375 380
Tyr Ala Leu Glu Asn Thr Met Ile Phe Asn Ser Gln Pro Ala Asn Pro
385 390 395 400
Gly Thr Thr Ala Thr Tyr Leu Glu Gly Asn Met Leu Ile Thr Ser Glu
405 410 415
Ser Glu Thr Gln Pro Val Asn Arg Val Ala Tyr Asn Val Gly Gly Gln
420 425 430
Met Ala Thr Asn Asn Gln Ser Ser Thr Thr Ala Pro Ala Thr Gly Thr
435 440 445
Tyr Asn Leu Gln Glu Ile Val Pro Gly Ser Val Trp Met Glu Arg Asp
450 455 460
Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro Glu Thr Gly Ala
465 470 475 480
His Phe His Pro Ser Pro Ala Met Gly Gly Phe Gly Leu Lys His Pro
485 490 495
Pro Pro Met Met Leu Ile Lys Asn Thr Pro Val Pro Gly Asn Ile Thr
500 505 510
Ser Phe Ser Asp Val Pro Val Ser Ser Phe Ile Thr Gln Tyr Ser Thr
515 520 525
Gly Gln Val Thr Val Glu Met Glu Trp Glu Leu Lys Lys Glu Asn Ser
530 535 540
Lys Arg Trp Asn Pro Glu Ile Gln Tyr Thr Asn Asn Tyr Asn Asp Pro
545 550 555 560
Gln Phe Val Asp Phe Ala Pro Asp Ser Thr Gly Glu Tyr Arg Thr Thr
565 570 575
Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg Pro Leu
580 585

<210> 6
<211> 532
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =

synthetic construct

<400> 6

Met Ser Ala Gly Gly Gly Gly Pro Leu Gly Asp Asn Asn Gln Gly Ala
1   5   10   15
Asp Gly Val Gly Asn Ala Ser Gly Asp Trp His Cys Asp Ser Thr Trp
            20   25   30
Met Gly Asp Arg Val Val Thr Lys Ser Thr Arg Thr Trp Val Leu Pro
        35   40   45

Ser Tyr Asn Asn His Gln Tyr Arg Glu Ile Lys Ser Gly Ser Val Asp
    50   55   60
Gly Ser Asn Ala Asn Ala Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr
65   70   75   80
Phe Asp Phe Asn Arg Phe His Ser His Trp Ser Pro Arg Asp Trp Gln
                85   90   95
Arg Leu Ile Asn Asn Tyr Trp Gly Phe Arg Pro Arg Ser Leu Arg Val
            100   105   110
Lys Ile Phe Asn Ile Gln Val Lys Glu Val Thr Val Gln Asp Ser Thr
        115   120   125
Thr Thr Ile Ala Asn Asn Leu Thr Ser Thr Val Gln Val Phe Thr Asp
    130   135   140
Asp Asp Tyr Gln Leu Pro Tyr Val Val Gly Asn Gly Thr Glu Gly Cys
145   150   155   160
Leu Pro Ala Phe Pro Pro Gln Val Phe Thr Leu Pro Gln Tyr Gly Tyr
                165   170   175
Ala Thr Leu Asn Arg Asp Asn Thr Glu Asn Pro Thr Glu Arg Ser Ser
            180   185   190
Phe Phe Cys Leu Glu Tyr Phe Pro Ser Lys Met Leu Arg Thr Gly Asn
        195   200   205
Asn Phe Glu Phe Thr Tyr Asn Phe Glu Glu Val Pro Phe His Ser Ser
    210   215   220
Phe Ala Pro Ser Gln Asn Leu Phe Lys Leu Ala Asn Pro Leu Val Asp
225   230   235   240
Gln Tyr Leu Tyr Arg Phe Val Ser Thr Asn Asn Thr Gly Gly Val Gln
                245   250   255
Phe Asn Lys Asn Leu Ala Gly Arg Tyr Ala Asn Thr Tyr Lys Asn Trp
            260   265   270
Phe Pro Gly Pro Met Gly Arg Thr Gln Gly Trp Asn Leu Gly Ser Gly
        275   280   285
Val Asn Arg Ala Ser Val Ser Ala Phe Ala Thr Thr Asn Arg Met Glu
    290   295   300
Leu Glu Gly Ala Ser Tyr Gln Val Pro Pro Gln Pro Asn Gly Met Thr
305   310   315   320
Asn Asn Leu Gln Gly Ser Asn Thr Tyr Ala Leu Glu Asn Thr Met Ile
                325   330   335
Phe Asn Ser Gln Pro Ala Asn Pro Gly Thr Thr Ala Thr Tyr Leu Glu
            340   345   350
Gly Asn Met Leu Ile Thr Ser Glu Ser Glu Thr Gln Pro Val Asn Arg
        355   360   365
Val Ala Tyr Asn Val Gly Gly Gln Met Ala Thr Asn Asn Gln Ser Ser

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    370                 375                 380
Thr Thr Ala Pro Ala Thr Gly Thr Tyr Asn Leu Gln Glu Ile Val Pro
385                 390                 395                 400
Gly Ser Val Trp Met Glu Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp
                405                 410                 415
Ala Lys Ile Pro Glu Thr Gly Ala His Phe His Pro Ser Pro Ala Met
            420                 425                 430
Gly Gly Phe Gly Leu Lys His Pro Pro Pro Met Met Leu Ile Lys Asn
        435                 440                 445
Thr Pro Val Pro Gly Asn Ile Thr Ser Phe Ser Asp Val Pro Val Ser
    450                 455                 460
Ser Phe Ile Thr Gln Tyr Ser Thr Gly Gln Val Thr Val Glu Met Glu
465                 470                 475                 480
Trp Glu Leu Lys Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Ile Gln
                485                 490                 495
Tyr Thr Asn Asn Tyr Asn Asp Pro Gln Phe Val Asp Phe Ala Pro Asp
            500                 505                 510
Ser Thr Gly Glu Tyr Arg Thr Thr Arg Pro Ile Gly Thr Arg Tyr Leu
        515                 520                 525
Thr Arg Pro Leu
    530
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<210> 7
<211> 2307
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
synthetic construct

<400> 7

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aggctctcat ttgttcccga gacgcctcgc agttcagacg tgactgttga tcccgctcct      60
ctgcgaccgc tcaattggaa ttcaagtaaa taaagcgagt agtcatgtct tttgttgatc     120
accctccaga ttggttggaa gaagttggtg aaggtcttcg cgagtttttg ggccttgaag     180
cgggcccacc gaaaccaaaa cccaatcagc agcatcaaga tcaagcccgt ggtcttgtgc     240
tgcctggtta taactatctc ggacccggaa acggtctcga tcgaggagag cctgtcaaca     300
gggcagacga ggtcgcgcga gagcacgaca tctcgtacaa cgagcagctt gaggcgggag     360
acaaccccta cctcaagtac aaccacgcgg acgccgagtt tcaggagaag ctcgccgacg     420
acacatcctt cggggggaaac ctcggaaagg cagtctttca ggccaagaaa agggttctcg     480
aacctttttgg cctggttgaa gagggtgcta agacggcccc taccggaaag cggatagacg     540
accactttcc aaaaagaaag aaggctcgga ccgaagagga ctccaagcct tccacctcgt     600
cagacgccga agctggaccc agcggatccc agcagctgca aatcccagcc caaccagcct     660
caagtttggg agctgataca atgtctgcgg gaggtggcgg cccattgggc gacaataacc     720
aaggtgccga tggagtgggc aatgcctcgg gagattggca ttgcgattcc acgtggatgg     780
gggacagagt cgtcaccaag tccacccgaa cctgggtgct gcccagctac aacaaccacc     840
agtaccgaga gatcaaaagc ggctccgtcg acggaagcaa cgccaacgcc tactttggat     900
acagcacccc ctgggggtac tttgacttta accgcttcca cagccactgg agcccccgag     960
actggcaaag actcatcaac aactactggg gcttcagacc ccggtccctc agagtcaaaa    1020
tcttcaacat tcaagtcaaa gaggtcacgg tgcaggactc caccaccacc atcgccaaca    1080
acctcacctc caccgtccaa gtgtttacgg acgacgacta ccagctgccc tacgtcgtcg    1140
```

```
gcaacgggac cgagggatgc ctgccggcct tccctccgca ggtctttacg ctgccgcagt    1200
acggttacgc gacgctgaac cgcgacaaca cagaaaatcc caccgagagg agcagcttct    1260
tctgcctaga gtactttccc agcaagatgc tgagaacggg caacaacttt gagtttacct    1320
acaactttga ggaggtgccc ttccactcca gcttcgctcc cagtcagaac ctgttcaagc    1380
tggccaaccc gctggtggac cagtacttgt accgcttcgt gagcacaaat aacactggcg    1440
gagtccagtt caacaagaac ctggccggga gatacgccaa cacctacaaa aactggttcc    1500
cggggcccat gggccgaacc cagggctgga acctgggctc cggggtcaac cgcgccagtg    1560
tcagcgcctt cgccacgacc aataggatgg agctcgaggg cgcgagttac caggtgcccc    1620
cgcagccgaa cggcatgacc aacaacctcc agggcagcaa cacctatgcc ctggagaaca    1680
ctatgatctt caacagccag ccggcgaacc cgggcaccac cgccacgtac ctcgagggca    1740
acatgctcat caccagcgag agcgagacgc agccggtgaa ccgcgtggcg tacaacgtcg    1800
gcgggcagat ggccaccaac aaccagagct ccaccactgc cccgcgacc ggcacgtaca    1860
acctccagga aatcgtgccc ggcagcgtgt ggatggagag ggacgtgtac ctccaaggac    1920
ccatctgggc caagatccca gagacggggg cgcactttca cccctctccg gccatgggcg    1980
gattcggact caaacaccca ccgcccatga tgctcatcaa gaacacgcct gtgcccggaa    2040
atatcaccag cttctcggac gtgcccgtca gcagcttcat cacccagtac agcaccgggc    2100
aggtcaccgt ggagatggag tgggagctca agaaggaaaa ctccaagagg tggaacccag    2160
agatccagta cacaaacaac tacaacgacc cccagtttgt ggactttgcc ccggacagca    2220
ccggggaata cagaaccacc agacctatcg gaacccgata ccttacccga cccctttaac    2280
ccattcatgt cgcataccct caataaa                                        2307
```

<210> 8
<211> 2264
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
synthetic construct

<400> 8

```
aggctctcat ttgttcccga gacgcctcgc agttcagacg tgactgttga tcccgctcct      60
ctgcgaccgc tcaattggaa ttcaagattg gttggaagaa gttggtgaag gtcttcgcga     120
gtttttgggc cttgaagcgg gcccaccgaa accaaaaccc aatcagcagc atcaagatca     180
agcccgtggt cttgtgctgc ctggttataa ctatctcgga cccggaaacg gtctcgatcg     240
aggagagcct gtcaacaggg cagacgaggt cgcgcgagag cacgacatct cgtacaacga     300
gcagcttgag gcgggagaca acccctacct caagtacaac cacgcggacg ccgagtttca     360
ggagaagctc gccgacgaca catccttcgg gggaaacctc ggaaaggcag tctttcaggc     420
caagaaaagg gttctcgaac cttttggcct ggttgaagag ggtgctaaga cggcccctac     480
cggaaagcgg atagacgacc actttccaaa aagaaagaag gctcggaccg aagaggactc     540
caagccttcc acctcgtcag acgccgaagc tggacccagc ggatcccagc agctgcaaat     600
cccagcccaa ccagcctcaa gtttgggagc tgatacaatg tctgcgggag gtggcggccc     660
attgggcgac aataaccaag gtgccgatgg agtgggcaat gcctcgggag attggcattg     720
cgattccacg tggatggggg acagagtcgt caccaagtcc acccgaacct gggtgctgcc     780
cagctacaac aaccaccagt accgagagat caaaagcggc tccgtcgacg gaagcaacgc     840
caacgcctac tttggataca gcacccccctg ggggtacttt gactttaacc gcttccacag     900
ccactggagc cccgagact ggcaaagact catcaacaac tactggggct tcagaccccg     960
gtccctcaga gtcaaaatct tcaacattca agtcaaagag gtcacggtgc aggactccac    1020
caccaccatc gccaacaacc tcacctccac cgtccaagtg tttacggacg acgactacca    1080
gctgccctac gtcgtcggca acgggaccga gggatgcctg ccggccttcc ctccgcaggt    1140
ctttacgctg ccgcagtacg gttacgcgac gctgaaccgc gacaacacag aaaatcccac    1200
```

```
cgagaggagc agcttcttct gcctagagta ctttcccagc aagatgctga gaacgggcaa    1260
caactttgag tttacctaca actttgagga ggtgcccttc cactccagct tcgctcccag    1320
tcagaacctg ttcaagctgg ccaacccgct ggtggaccag tacttgtacc gcttcgtgag    1380
cacaaataac actggcggag tccagttcaa caagaacctg gccgggagat acgccaacac    1440
ctacaaaaac tggttcccgg ggcccatggg ccgaacccag ggctggaacc tgggctccgg    1500
ggtcaaccgc gccagtgtca gcgccttcgc cacgaccaat aggatggagc tcgagggcgc    1560
gagttaccag gtgcccccgc agccgaacgg catgaccaac aacctccagg gcagcaacac    1620
ctatgccctg gagaacacta tgatcttcaa cagccagccg gcgaacccgg gcaccaccgc    1680
cacgtacctc gagggcaaca tgctcatcac cagcgagagc gagacgcagc cggtgaaccg    1740
cgtggcgtac aacgtcggcg ggcagatggc caccaacaac cagagctcca ccactgcccc    1800
cgcgaccggc acgtacaacc tccaggaaat cgtgcccggc agcgtgtgga tggagaggga    1860
cgtgtacctc caaggaccca tctgggccaa gatcccagag acgggggcgc actttcaccc    1920
ctctccggcc atgggcggat tcggactcaa acacccaccg cccatgatgc tcatcaagaa    1980
cacgcctgtg cccggaaata tcaccagctt ctcggacgtg cccgtcagca gcttcatcac    2040
ccagtacagc accgggcagg tcaccgtgga gatggagtgg gagctcaaga aggaaaactc    2100
caagaggtgg aacccagaga tccagtacac aaacaactac aacgaccccc agtttgtgga    2160
ctttgccccg gacagcaccg ggaatacag aaccaccaga cctatcggaa cccgatacct     2220
tacccgaccc ctttaaccca ttcatgtcgc ataccctcaa taaa                     2264
```

<210> 9
<211> 2264
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
synthetic construct

<400> 9

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aggctctcat ttgttcccga gacgcctcgc agttcagacg tgactgttga tcccgctcct      60
ctgcgaccgc tcaattggaa ttcaagattg gttggaagaa gttggtgaag gtcttcgcga     120
gtttttgggc cttgaagcgg gcccaccgaa accaaaaccc aatcagcagc atcaagatca     180
agcccgtggt cttgtgctgc ctggttataa ctatctcgga cccggaaacg gtctcgatcg     240
aggagagcct gtcaacaggg cagacgaggt cgcgcgagag cacgacatct cgtacaacga     300
gcagcttgag gcgggagaca acccctacct caagtacaac cacgcggacg ccgagtttca     360
ggagaagctc gccgacgaca catccttcgg gggaaacctc ggaaaggcag tctttcaggc     420
caagaaaagg gttctcgaac cttttggcct ggttgaagag ggtgctaaga cggcccctac     480
cggaaagcgg atagacgacc actttccaaa aagaaagaag gctcggaccg aagaggactc     540
caagccttcc acctcgtcag acgccgaagc tggacccagc ggatcccagc agctgcaaat     600
cccagcccaa ccagcctcaa gtttgggagc tgatacaatg tctgcgggag gtggcggccc     660
attgggcgac aataaccaag gtgccgatgg agtgggcaat gcctcgggag attggcattg     720
cgattccacg tggatggggg acagagtcgt caccaagtcc acccgaacct gggtgctgcc     780
cagctacaac aaccaccagt accgagagat caaaagcggc tccgtcgacg gaagcaacgc     840
caacgcctac tttggataca gcaccccctg ggggtacttt gactttaacc gcttccacag     900
ccactggagc ccccgagact ggcaaagact catcaacaac tactggggct tcagaccccg     960
gtccctcaga gtcaaaatct tcaacattca agtcaaagag gtcacggtgc aggactccac    1020
caccaccatc gccaacaacc tcacctccac cgtccaagtg tttacggacg acgactacca    1080
gctgccctac gtcgtcggca acgggaccga gggatgcctg ccggccttcc ctccgcaggt    1140
ctttacgctg ccgcagtacg gttacgcgac gctgaaccgc gacaacacag aaaatcccac    1200
cgagaggagc agcttcttct gcctagagta ctttcccagc aagatgctga gaacgggcaa    1260
caactttgag tttacctaca actttgagga ggtgcccttc cactccagct tcgctcccag    1320
```

```
tcagaacctg ttcaagctgg ccaacccgct ggtggaccag tacttgtacc gcttcgtgag    1380
cacaaataac actggcggag tccagttcaa caagaacctg gccgggagat acgccaacac    1440
ctacaaaaac tggttcccgg ggcccatggg ccgaacccag ggctggaacc tgggctccgg    1500
ggtcaaccgc gccagtgtca gcgccttcgc cacgaccaat aggatggagc tcgagggcgc    1560
gagttaccag gtgcccccgc agccgaacgg catgaccaac aacctccagg gcagcaacac    1620
ctatgccctg gagaacacta tgatcttcaa cagccagccg gcgaacccgg gcaccaccgc    1680
cacgtacctc gagggcaaca tgctcatcac cagcgagagc gagacgcagc cggtgaaccg    1740
cgtggcgtac aacgtcggcg ggcagatggc caccaacaac cagagctcca ccactgcccc    1800
cgcgaccggc acgtacaacc tccaggaaat cgtgcccggc agcgtgtgga tggagaggga    1860
cgtgtacctc caaggaccca tctgggccaa gatcccagag acgggggcgc actttcaccc    1920
ctctccggcc atgggcggat tcggactcaa acacccaccg cccatgatgc tcatcaagaa    1980
cacgcctgtg cccggaaata tcaccagctt ctcggacgtg cccgtcagca gcttcatcac    2040
ccagtacagc accgggcagg tcaccgtgga gatggagtgg gagctcaaga aggaaaactc    2100
caagaggtgg aacccagaga tccagtacac aaacaactac aacgaccccc agtttgtgga    2160
ctttgccccg gacagcaccg gggaatacag aaccaccaga cctatcggaa cccgatacct    2220
tacccgaccc ctttaaccca ttcatgtcgc ataccctcaa taaa                     2264
```

<210> 10
<211> 1292
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
synthetic construct

<400> 10

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agcgcaaacg gctcgtcgcg cagtttctgg cagaatcctc gcagcgctcg caggaggcgg      60
cttcgcagcg tgagttctcg gctgacccgg tcatcaaaag caagacttcc cagaaataca     120
tggcgctcgt caactggctc gtggagcacg gcatcacttc cgagaagcag tggatccagg     180
aaaatcagga gagctacctc tccttcaact ccaccggcaa ctctcggagc cagatcaagg     240
ccgcgctcga caacgcgacc aaaattatga gtctgacaaa aagcgcggtg gactacctcg     300
tggggagctc cgttcccgag gacatttcaa aaaacagaat ctggcaaatt tttgagatga     360
atggctacga cccggcctac gcgggatcca tcctctacgg ctggtgtcag cgctccttca     420
acaagaggaa caccgtctgg ctctacggac ccgccacgac cggcaagacc aacatcgcgg     480
aggccatcgc ccacactgtg ccctttttacg gctgcgtgaa ctggaccaat gaaaactttc     540
cctttaatga ctgtgtggac aaaatgctca tttggtggga ggagggaaag atgaccaaca     600
aggtggttga atccgccaag gccatcctgg ggggctcaaa ggtgcgggtc gatcagaaat     660
gtaaatcctc tgttcaaatt gattctaccc ctgtcattgt aacttccaat acaaacatgt     720
gtgtggtggt ggatgggaat tccacgacct ttgaacacca gcagccgctg gaggaccgca     780
tgttcaaatt tgaactgact aagcggctcc cgccagattt tggcaagatt actaagcagg     840
aagtcaagga ctttttttgct tggcaaaggg tcaatcaggt gccggtgact cacgagttta     900
aagttcccag ggaattggcg ggaactaaag gggcggagaa atctctaaaa cgcccactgg     960
gtgacgtcac caatactagc tataaaagtc tggagaagcg ggccaggctc tcatttgttc    1020
ccgagacgcc tcgcagttca gacgtgactg ttgatcccgc tcctctgcga ccgctcaatt    1080
ggaattcaag gtatgattgc aaatgtgact atcatgctca atttgacaac atttctaaca    1140
aatgtgatga atgtgaatat ttgaatcggg gcaaaaatgg atgtatctgt cacaatgtaa    1200
ctcactgtca aatttgtcat gggattcccc cctgggaaaa ggaaaacttg tcagattttg    1260
gggattttga cgatgccaat aaagaacagt aa                                   1292
```

<210> 11

<211> 1870
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
synthetic construct

<400> 11

```
attctttgct ctggactgct agaggaccct cgctgccatg gctaccttct atgaagtcat      60
tgttcgcgtc ccatttgacg tggaggaaca tctgcctgga atttctgaca gctttgtgga     120
ctgggtaact ggtcaaattt gggagctgcc tccagagtca gatttaaatt tgactctggt     180
tgaacagcct cagttgacgg tggctgatag aattcgccgc gtgttcctgt acgagtggaa     240
caaattttcc aagcaggagt ccaaattctt tgtgcagttt gaaaagggat ctgaatattt     300
tcatctgcac acgcttgtgg agacctccgg catctcttcc atggtcctcg gccgctacgt     360
gagtcagatt cgcgcccagc tggtgaaagt ggtcttccag ggaattgaac cccagatcaa     420
cgactgggtc gccatcacca aggtaaagaa gggcggagcc aataaggtgg tggattctgg     480
gtatattccc gcctacctgc tgccgaaggt ccaaccggag cttcagtggg cgtggacaaa     540
cctggacgag tataaattgg ccgccctgaa tctggaggag cgcaaacggc tcgtcgcgca     600
gtttctggca gaatcctcgc agcgctcgca ggaggcggct tcgcagcgtg agttctcggc     660
tgacccggtc atcaaaagca agacttccca gaaatacatg gcgctcgtca actggctcgt     720
ggagcacggc atcacttccg agaagcagtg gatccaggaa aatcaggaga gctacctctc     780
cttcaactcc accggcaact ctcggagcca gatcaaggcc gcgctcgaca acgcgaccaa     840
aattatgagt ctgacaaaaa gcgcggtgga ctacctcgtg gggagctccg ttcccgagga     900
catttcaaaa aacagaatct ggcaaatttt tgagatgaat ggctacgacc cggcctacgc     960
gggatccatc ctctacggct ggtgtcagcg ctccttcaac aagaggaaca ccgtctggct    1020
ctacggaccc gccacgaccg gcaagaccaa catcgcggag gccatcgccc acactgtgcc    1080
cttttacggc tgcgtgaact ggaccaatga aaactttccc tttaatgact gtgtggacaa    1140
aatgctcatt tggtgggagg agggaaagat gaccaacaag gtggttgaat ccgccaaggc    1200
catcctgggg ggctcaaagg tgcgggtcga tcagaaatgt aaatcctctg ttcaaattga    1260
ttctacccct gtcattgtaa cttccaatac aaacatgtgt gtggtggtgg atgggaattc    1320
cacgaccttt gaacaccagc agccgctgga ggaccgcatg ttcaaatttg aactgactaa    1380
gcggctcccg ccagattttg gcaagattac taagcaggaa gtcaaggact tttttgcttg    1440
ggcaaaggtc aatcaggtgc cggtgactca cgagtttaaa gttcccaggg aattggcggg    1500
aactaaaggg gcggagaaat ctctaaaacg cccactgggt gacgtcacca atactagcta    1560
taaaagtctg gagaagcggg ccaggctctc atttgttccc gagacgcctc gcagttcaga    1620
cgtgactgtt gatcccgctc ctctgcgacc gctcaattgg aattcaaggt atgattgcaa    1680
atgtgactat catgctcaat ttgacaacat ttctaacaaa tgtgatgaat gtgaatattt    1740
gaatcggggc aaaaatggat gtatctgtca caatgtaact cactgtcaaa tttgtcatgg    1800
gattcccccc tgggaaaagg aaaacttgtc agattttggg gattttgacg atgccaataa    1860
agaacagtaa                                                           1870
```

<210> 12
<211> 330
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
synthetic construct

<400> 12
Met Ala Leu Val Asn Trp Leu Val Glu His Gly Ile Thr Ser Glu Lys
1 5 10 15
Gln Trp Ile Gln Glu Asn Gln Glu Ser Tyr Leu Ser Phe Asn Ser Thr
20 25 30
Gly Asn Ser Arg Ser Gln Ile Lys Ala Ala Leu Asp Asn Ala Thr Lys
35 40 45
Ile Met Ser Leu Thr Lys Ser Ala Val Asp Tyr Leu Val Gly Ser Ser
50 55 60
Val Pro Glu Asp Ile Ser Lys Asn Arg Ile Trp Gln Ile Phe Glu Met
65 70 75 80
Asn Gly Tyr Asp Pro Ala Tyr Ala Gly Ser Ile Leu Tyr Gly Trp Cys
85 90 95
Gln Arg Ser Phe Asn Lys Arg Asn Thr Val Trp Leu Tyr Gly Pro Ala
100 105 110
Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Thr Val Pro
115 120 125
Phe Tyr Gly Cys Val Asn Trp Thr Asn Glu Asn Phe Pro Phe Asn Asp
130 135 140
Cys Val Asp Lys Met Leu Ile Trp Trp Glu Glu Gly Lys Met Thr Asn
145 150 155 160
Lys Val Val Glu Ser Ala Lys Ala Ile Leu Gly Gly Ser Lys Val Arg
165 170 175
Val Asp Gln Lys Cys Lys Ser Ser Val Gln Ile Asp Ser Thr Pro Val
180 185 190
Ile Val Thr Ser Asn Thr Asn Met Cys Val Val Val Asp Gly Asn Ser
195 200 205
Thr Thr Phe Glu His Gln Gln Pro Leu Glu Asp Arg Met Phe Lys Phe
210 215 220
Glu Leu Thr Lys Arg Leu Pro Pro Asp Phe Gly Lys Ile Thr Lys Gln
225 230 235 240
Glu Val Lys Asp Phe Phe Ala Trp Ala Lys Val Asn Gln Val Pro Val
245 250 255
Thr His Glu Phe Lys Val Pro Arg Glu Leu Ala Gly Thr Lys Gly Ala
260 265 270
Glu Lys Ser Leu Lys Arg Pro Leu Gly Asp Val Thr Asn Thr Ser Tyr
275 280 285
Lys Ser Leu Glu Lys Arg Ala Arg Leu Ser Phe Val Pro Glu Thr Pro
290 295 300
Arg Ser Ser Asp Val Thr Val Asp Pro Ala Pro Leu Arg Pro Leu Asn
305 310 315 320
Trp Asn Ser Arg Leu Val Gly Arg Ser Trp
325 330

<210> 13
<211> 1115
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =

synthetic construct

<400> 13
aggagcgcaa acggctcgtc gcgcagtttc tggcagaatc ctcgcagcgc tcgcaggagg 60
cggcttcgca gcgtgagttc tcggctgacc cggtcatcaa aagcaagact tcccagaaat 120
acatggcgct cgtcaactgg ctcgtggagc acggcatcac ttccgagaag cagtggatcc 180
aggaaaatca ggagagctac ctctccttca actccaccgg caactctcgg agccagatca 240
aggccgcgct cgacaacgcg accaaaatta tgagtctgac aaaaagcgcg gtggactacc 300
tcgtggggag ctccgttccc gaggacattt caaaaaacag aatctggcaa atttttgaga 360
tgaatggcta cgacccggcc tacgcgggat ccatcctcta cggctggtgt cagcgctcct 420
tcaacaagag gaacaccgtc tggctctacg gacccgccac gaccggcaag accaacatcg 480
cggaggccat cgcccacact gtgccctttt acggctgcgt gaactggacc aatgaaaact 540
ttccctttaa tgactgtgtg gacaaaatgc tcatttggtg ggaggaggga aagatgacca 600
acaaggtggt tgaatccgcc aaggccatcc tgggggctc aaaggtgcgg gtcgatcaga 660
aatgtaaatc ctctgttcaa attgattcta cccctgtcat tgtaacttcc aatacaaaca 720
tgtgtgtggt ggtggatggg aattccacga cctttgaaca ccagcagccg ctggaggacc 780
gcatgttcaa atttgaactg actaagcggc tcccgccaga ttttggcaag attactaagc 840
aggaagtcaa ggactttttt gcttgggcaa aggtcaatca ggtgccggtg actcacgagt 900
ttaaagttcc cagggaattg gcgggaacta aaggggcgga gaaatctcta aaacgcccac 960
tgggtgacgt caccaatact agctataaaa gtctggagaa gcgggccagg ctctcatttg 1020
ttcccgagac gcctcgcagt tcagacgtga ctgttgatcc cgctcctctg cgaccgctca 1080
attggaattc aagattggtt ggaagaagtt ggtga 1115

<210> 14
<211> 550
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
synthetic construct

<400> 14
Met Ala Thr Phe Tyr Glu Val Ile Val Arg Val Pro Phe Asp Val Glu
1 5 10 15
Glu His Leu Pro Gly Ile Ser Asp Ser Phe Val Asp Trp Val Thr Gly
20 25 30
Gln Ile Trp Glu Leu Pro Pro Glu Ser Asp Leu Asn Leu Thr Leu Val
35 40 45
Glu Gln Pro Gln Leu Thr Val Ala Asp Arg Ile Arg Arg Val Phe Leu
50 55 60
Tyr Glu Trp Asn Lys Phe Ser Lys Gln Glu Ser Lys Phe Phe Val Gln
65 70 75 80
Phe Glu Lys Gly Ser Glu Tyr Phe His Leu His Thr Leu Val Glu Thr
85 90 95
Ser Gly Ile Ser Ser Met Val Leu Gly Arg Tyr Val Ser Gln Ile Arg
100 105 110
Ala Gln Leu Val Lys Val Val Phe Gln Gly Ile Glu Pro Gln Ile Asn
115 120 125
Asp Trp Val Ala Ile Thr Lys Val Lys Lys Gly Gly Ala Asn Lys Val
130 135 140

Val Asp Ser Gly Tyr Ile Pro Ala Tyr Leu Leu Pro Lys Val Gln Pro
145 150 155 160
Glu Leu Gln Trp Ala Trp Thr Asn Leu Asp Glu Tyr Lys Leu Ala Ala
165 170 175
Leu Asn Leu Glu Glu Arg Lys Arg Leu Val Ala Gln Phe Leu Ala Glu
180 185 190
Ser Ser Gln Arg Ser Gln Glu Ala Ala Ser Gln Arg Glu Phe Ser Ala
195 200 205

Asp Pro Val Ile Lys Ser Lys Thr Ser Gln Lys Tyr Met Ala Leu Val
210 215 220
Asn Trp Leu Val Glu His Gly Ile Thr Ser Glu Lys Gln Trp Ile Gln
225 230 235 240
Glu Asn Gln Glu Ser Tyr Leu Ser Phe Asn Ser Thr Gly Asn Ser Arg
245 250 255
Ser Gln Ile Lys Ala Ala Leu Asp Asn Ala Thr Lys Ile Met Ser Leu
260 265 270
Thr Lys Ser Ala Val Asp Tyr Leu Val Gly Ser Ser Val Pro Glu Asp
275 280 285
Ile Ser Lys Asn Arg Ile Trp Gln Ile Phe Glu Met Asn Gly Tyr Asp
290 295 300
Pro Ala Tyr Ala Gly Ser Ile Leu Tyr Gly Trp Cys Gln Arg Ser Phe
305 310 315 320
Asn Lys Arg Asn Thr Val Trp Leu Tyr Gly Pro Ala Thr Thr Gly Lys
325 330 335
Thr Asn Ile Ala Glu Ala Ile Ala His Thr Val Pro Phe Tyr Gly Cys
340 345 350
Val Asn Trp Thr Asn Glu Asn Phe Pro Phe Asn Asp Cys Val Asp Lys
355 360 365
Met Leu Ile Trp Trp Glu Glu Gly Lys Met Thr Asn Lys Val Val Glu
370 375 380
Ser Ala Lys Ala Ile Leu Gly Gly Ser Lys Val Arg Val Asp Gln Lys
385 390 395 400
Cys Lys Ser Ser Val Gln Ile Asp Ser Thr Pro Val Ile Val Thr Ser
405 410 415
Asn Thr Asn Met Cys Val Val Val Asp Gly Asn Ser Thr Thr Phe Glu
420 425 430
His Gln Gln Pro Leu Glu Asp Arg Met Phe Lys Phe Glu Leu Thr Lys
435 440 445
Arg Leu Pro Pro Asp Phe Gly Lys Ile Thr Lys Gln Glu Val Lys Asp
450 455 460
Phe Phe Ala Trp Ala Lys Val Asn Gln Val Pro Val Thr His Glu Phe
465 470 475 480
Lys Val Pro Arg Glu Leu Ala Gly Thr Lys Gly Ala Glu Lys Ser Leu
485 490 495
Lys Arg Pro Leu Gly Asp Val Thr Asn Thr Ser Tyr Lys Ser Leu Glu
500 505 510
Lys Arg Ala Arg Leu Ser Phe Val Pro Glu Thr Pro Arg Ser Ser Asp
515 520 525
Val Thr Val Asp Pro Ala Pro Leu Arg Pro Leu Asn Trp Asn Ser Arg
530 535 540

Leu Val Gly Arg Ser Trp
545 550

<210> 15
<211> 1690
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
synthetic construct

<400> 15
attctttgct ctggactgct agaggaccct cgctgccatg gctaccttct atgaagtcat 60
tgttcgcgtc ccatttgacg tggaggaaca tctgcctgga atttctgaca gctttgtgga 120
ctgggtaact ggtcaaattt gggagctgcc tccagagtca gatttaaatt tgactctggt 180
tgaacagcct cagttgacgg tggctgatag aattcgccgc gtgttcctgt acgagtggaa 240
caaattttcc aagcaggagt ccaaattctt tgtgcagttt gaaaagggat ctgaatattt 300
tcatctgcac acgcttgtgg agacctccgg catctcttcc atggtcctcg gccgctacgt 360
gagtcagatt cgcgcccagc tggtgaaagt ggtcttccag ggaattgaac cccagatcaa 420
cgactgggtc gccatcacca aggtaaagaa gggcggagcc aataaggtgg tggattctgg 480
gtatattccc gcctacctgc tgccgaaggt ccaaccggag cttcagtggg cgtggacaaa 540
cctggacgag tataaattgg ccgccctgaa tctggaggag cgcaaacggc tcgtcgcgca 600
gtttctggca gaatcctcgc agcgctcgca ggaggcggct tcgcagcgtg agttctcggc 660
tgacccggtc atcaaaagca agacttccca gaaatacatg gcgctcgtca actggctcgt 720
ggagcacggc atcacttccg agaagcagtg gatccaggaa aatcaggaga gctacctctc 780
cttcaactcc accggcaact ctcggagcca gatcaaggcc gcgctcgaca acgcgaccaa 840
aattatgagt ctgacaaaaa gcgcggtgga ctacctcgtg gggagctccg ttcccgagga 900
catttcaaaa aacagaatct ggcaaatttt tgagatgaat ggctacgacc cggcctacgc 960
gggatccatc ctctacggct ggtgtcagcg ctccttcaac aagaggaaca ccgtctggct 1020
ctacggaccc gccacgaccg gcaagaccaa catcgcggag gccatcgccc acactgtgcc 1080
cttttacggc tgcgtgaact ggaccaatga aaactttccc tttaatgact gtgtggacaa 1140
aatgctcatt tggtgggagg agggaaagat gaccaacaag gtggttgaat ccgccaaggc 1200
catcctgggg ggctcaaagg tgcgggtcga tcagaaatgt aaatcctctg ttcaaattga 1260
ttctacccct gtcattgtaa cttccaatac aaacatgtgt gtggtggtgg atgggaattc 1320
cacgaccttt gaacaccagc agccgctgga ggaccgcatg ttcaaatttg aactgactaa 1380
gcggctcccg ccagattttg gcaagattac taagcaggaa gtcaaggact tttttgcttg 1440
ggcaaaggtc aatcaggtgc cggtgactca cgagtttaaa gttcccaggg aattggcggg 1500
aactaaaggg gcggagaaat ctctaaaacg cccactgggt gacgtcacca atactagcta 1560
taaaagtctg gagaagcggg ccaggctctc atttgttccc gagacgcctc gcagttcaga 1620
cgtgactgtt gatcccgctc ctctgcgacc gctcaattgg aattcaagat tggttggaag 1680
aagttggtga 1690

<210> 16
<211> 145
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
synthetic construct

<400> 16
ccatcaccaa ggtaaagaag ggcggagcca ataaggtggt ggattctggg tatattcccg 60
cctacctgct gccgaaggtc caaccggagc ttcagtgggc gtggacaaac ctggacgagt 120
ataaattggc cgccctgaat ctgga 145

<210> 17
<211> 174
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
synthetic construct

<400> 17
taagcaggaa gtcaaggact tttttgcttg ggcaaaggtc aatcaggtgc cggtgactca 60
cgagtttaaa gttcccaggg aattggcggg aactaaaggg gcggagaaat ctctaaaacg 120
cccactgggt gacgtcacca atactagcta taaaagtctg gagaagcggg ccag 174

<210> 18
<211> 187
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
synthetic construct

<400> 18
cactctcaag caaggggggtt ttgtaagcag tgatgtcata atgatgtaat gcttattgtc 60
acgcgatagt taatgattaa cagtcatgtg atgtgtttta tccaatagga agaaagcgcg 120
cgtatgagtt ctcgcgagac ttccggggta taaaagaccg agtgaacgag cccgccgcca 180
ttctttg 187

<210> 19
<211> 168
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
synthetic construct

<400> 19
aaacctcctt gcttgagagt gtggcactct ccccccctgtc gcgttcgctc gctcgctggc 60
tcgtttgggg gggtggcagc tcaaagagct gccagacgac ggccctctgg ccgtcgcccc 120
cccaaacgag ccagcgagcg agcgaacgcg acagggggga gagtgcca 168

<210> 20
<211> 168
<212> DNA

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
synthetic construct

<400> 20
aaacctcctt gcttgagagt gtggcactct ccccсctgtc gcgttcgctc gctcgctggc 60
tcgtttgggg gggcgacggc cagagggccg tcgtctgccg gctctttgag ctgccacccc 120
cccaaacgag ccagcgagcg agcgaacgcg acaggggggа gagtgcca 168

<210> 21
<211> 8
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
synthetic construct

<400> 21
cggtgtga 8

<210> 22
<211> 8
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
synthetic construct

<400> 22
cggttgag 8

<210> 23
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/Note =
synthetic construct

<400> 23
caaaacctcc ttgcttgaga g 21